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EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

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LOCAL SPECIFIC THERAPY OF EXPERIMENTAL PNEUMOCOCCAL MENINGITIS.

I. Experimental Pneumococcal Meningitis in Rabbits.

By FRED W. STEWART, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 11 to 15.

(Received for publication, May 31, 1927.)

Pneumococcal meningitis is not a common disease, and yet a certain number of cases appears each year in most larger hospitals. The disease is quite uniformly a fatal one. Whereas occasionally descriptions of recovery appear in the literature, one is frequently left with the impression that diagnosis was either incomplete or incorrect in such instances. To all intents and purposes, therefore, the disease may be considered as having a mortality of nearly 100 per cent. Consequently, any treatment, drastic as it may at first appear, which offers hope of benefit to even a small number of these unfortunate patients, may be worthy of consideration.

The problem of advancing the therapy of pneumococcal meningitis has interested this laboratory beginning with the study of Lamar (1). Lamar's work was done long before our far fuller knowledge of the pneumococci was obtained. His study was therefore somewhat incomplete from our present day point of view. Lamar was impressed by the fact that the pneumococcus was bile-soluble. It occurred to him, therefore, to attempt to influence the course of experimental pneumococcal meningitis by the intrathecal administration of mixtures of immune serum, sodium oleate, and boric acid. He had already noted (2) that pneumococci treated with sodium oleate became more subject to autolysis and that they became at the same time susceptible to serum lysis. This serum lysis tended to be incomplete with normal serum, but when immune serum was employed, lysis was complete, no multiplication of organisms took place in the test-tube mixture, and an inoculated animal was protected from infection. However, the soap action was inhibited in the presence of protein, and Lamar developed a method of minimizing this protein inhibition by adding to the serum-sodium oleate an appropriate quantity of boric acid. A number of monkeys was infected

directly in the lumbar subarachnoid space with a culture isolated from a human case of pneumococcal meningitis; the organism was not typed; it had only moderate mouse virulence, but produced in the monkey a fatal meningitis, closely resembling pneumococcal meningitis in man, save that the course of the experimental disease was more rapid. Lamar found that repeated administration of mixtures of sodium oleate, immune serum, and boric acid would arrest established infections and lead to enduring recoveries.

The value of this treatment may be determined only from its employment in human cases of the disease, and apparently little benefit followed its use (Kolmer (3)). Nor in our opinion have subsequent developments in therapy, such as Kolmer (3) and Kolmer and Idzumi (4) advocate, namely, saline lavage, or combinations of saline lavage with the administration of the antibody solution of Huntoon, or with various chemotherapeutic agents given much promise of beneficial results in man. Kolmer's results will be more fully discussed in a subsequent paper.

In view of all these facts, it was decided to reinvestigate pneumococcal meningitis. The first procedure consisted in a search for a satisfactory laboratory animal. Cats proved too resistant for our purposes; whereas with large doses of Type I pneumococci injected directly into the cisterna magna it was possible to produce acute leptomeningitis, the results were irregular and it was felt that it was not justifiable to draw conclusions from so resistant an animal. Attempts to produce the disease in guinea pigs were abandoned for similar reasons. The rabbit was at first reserved because of its high susceptibility, but was, nevertheless, next employed. In our experience the rabbit presents a difficult problem so far as highly virulent Type I pneumococcal infections are concerned in that, though extremely responsive to pneumococci, the production of meningeal disease in any way resembling that found in man was not readily attained.

If a normal rabbit receives Type I pneumococci in the cisterna magna, the development of septicemia is very rapid, and death is septicemic rather than meningeal. The cellular reactional processes common to human pneumococcal meningitis, as a rule, do not occur. Such, however, appears not to be the conclusion of Idzumi (5), for the latter reports vigorous leucocytic reactions due to Type I pneumococci in the rabbit meninges. Idzumi's pneumococci were apparently of low virulence, the mouse M.L.D. for his Type I being 0.001 cc. in 24 to 30 hours. It was employed in relatively enormous dosage—dosage at least 100 times as great as we have required to produce a fatal

septicemia without much meningeal reaction in a normal rabbit. In addition, Idzumi used a 24 hour culture, undoubtedly containing many damaged cocci. More recently pneumococcal meningitis has been studied in the rabbit by Untersteiner (6). The organism used was not typed, and we are therefore unable to analyze his results in terms of our own.

EXPERIMENTAL.

In studying pneumococcal meningitis of rabbits from the point of view of therapy, one is limited by several considerations: (1) the extreme susceptibility of the animal; (2) the invariable development of massive septicemia when virulent organisms are used—a septicemia which kills before the reactional process in the meninges develops to a sufficient extent to dignify it by the term "meningitis;" (3) the difficulty in lumbar punctures in the rabbit; (4) the limited working space for repeated injections into the cisterna magna resulting in frequent trauma; and (5) the rather soft consistency of the rabbit brain, rendering pressure deformation frequent.

It became apparent early that pneumococci injected into the rabbit's cisterna reached the blood stream in enormous numbers, beginning almost immediately and resulting in massive septicemia within so short a time as 5 hours after infection. Animals died within less than 24 hours, the anatomical cause of death being apparently intense edema and congestion of the lungs. Pneumococci could be demonstrated readily in the capillaries of all the large viscera (Figs. 1 and 2) and in blood smears. These findings led us to adopt methods of preventing the septicemia or of minimizing it to such an extent as to render it a negligible factor in the death of the rabbit. Naturally, recourse was had to partial immunization of the animals either passively, or actively. Rabbits were passively immunized by the intravenous administration, either just before cisternal infection, or a few hours previously. Usually 10 cc. of Type I antipneumococcus serum (therapeutic) was used. This serum was kindly supplied us by Dr. Augustus Wadsworth, of the Laboratory of the New York State Department of Health. Its potency was such that 0.2 cc. protected mice against simultaneous inoculation of 0.2 cc. of standard culture. Active immunity was established by one or two series of six intravenous

TABLE I.

No.	Type	Method of immunization	Infecting dose	Site of infection	Result of blood cultures					Duration of life	Post-mortem heart blood culture	Meningeal reaction
					1–1½ hrs.	2½–3 hrs.	5–5½ hrs.	24 hrs.	48 hrs.			
1	Actively immune	6 injections, 1 cc. vaccine, intravenously	0.5 cc.; 1:50, 18 hr. culture	Cistern	0	0	5	0	0	17 days	0	0
2	Control	None	0.5 cc.; 1:50, 18 hr. culture	"	25	370	∞			27 hrs.	+	+
3	Actively immune	6 injections, 1 cc. vaccine, intravenously	16,000,000, 18 hr. culture	"	0	0	0	0		32 days	0	0
4	Control	None	16,000,000, 18 hr. culture	"	0	0	0	4620		45 hrs.	+	++
5	Actively immune	12 injections, 1 cc. vaccine, intravenously	111,000,000, 18 hr. culture	"	60	810	4020			22 "	+	++++
6	Control	None	111,000,000, 18 hr. culture	"	31,800	∞	∞			22 "	+	+
7	Actively immune	12 injections, 1 cc. vaccine, intravenously	55,000,000, 18 hr. culture	"	0	0	Accidental death			5½ "	+	0
8	Control	None	55,000,000, 18 hr. culture	"	0	920	∞			26 "	+	+
9	Actively immune	12 injections, 1 cc. vaccine, intravenously	81,000,000, 18 hr. culture	"	20	165	375			22 "	+	++
10	Control	None	81,000,000, 18 hr. culture	"	530	∞	∞			22 "	+	++
11	Passively immune	5 cc. N.Y.S. serum intravenously	35,000,000, 18 hr. culture	"	20	535	900			22 "	+	++++

12	Passively immune	5 cc. N.Y.S. serum intravenously	35,000,000, 18 hr. culture	Cistern	0	750	4640	60		44 days	+	++
13	Control	None	35,000,000, 18 hr. culture	"	55	1600	42,840			22 "	+	+++
14	Passively immune	10 cc. N.Y.S. serum intravenously	26,000,000, 18 hr. culture	"	5	290	2180	60		46 "	+	+++
15	Actively immune	6 injections, 1 cc. vaccine, intravenously	26,000,000, 18 hr. culture	"	0	15	15			26 "	0	++++
16	Control	None	26,000,000, 18 hr. culture	"	2320	∞	∞			22 "	+	++
17	Passively immune	10 cc. N.Y.S. serum intravenously	13,000,000, 18 hr. culture	"	0	0	0			22 "	+	++++
18	Passively immune	10 cc. N.Y.S. serum intravenously	13,000,000, 18 hr. culture	"	30	750	1920			22 "	+	++++
19	Control	None	13,000,000, 18 hr. culture	"	+ (Contam.)	10,800	∞			22 "	No autopsy	
20	Passively immune	10 cc. N.Y.S. serum intravenously	9,000,000, 18 hr. culture	"		135	4920	1060		45 "	+	++
21	Passively immune	10 cc. N.Y.S. serum intravenously	4,500,000, 18 hr. culture	"		56	510	10	640	72 "	0	++
22	Passively immune	10 cc. N.Y.S. serum intravenously	1,000,000, 18 hr. culture	"		80	265	0	780	72 "	0	++++
23	Control	None	1,000,000, 18 hr. culture	"		1740	12,280	41,600		45 "	+	++
24	Passively immune	10 cc. N.Y.S. serum intravenously	5,000,000, 18 hr. culture	"		0	175	0		70 "	0	+++
25	Passively immune	10 cc. N.Y.S. serum intravenously	5,000,000, 18 hr. culture	"		0	5	0		24 "	0	++++
26	Control	None	5,000,000, 18 hr. culture	"		11,360	16,280			Hemorrhage 46 hrs.	+	+

TABLE I—*Concluded.*

No.	Type	Method of immunization	Infecting dose	Site of infection	Result of blood cultures					Duration of life	Post-mortem heart blood culture	Meningeal reaction
					1–1½ hrs.	2½–3 hrs.	5–5½ hrs.	24 hrs.	48 hrs.			
27	Passively immune	10 cc. N.Y.S. serum intravenously	6,000,000, 18 hr. culture	Ear vein		0	5	0		Lived		
28	Passively immune	10 cc. N.Y.S. serum intravenously	6,000,000, 18 hr. culture	" "		0	0	0		"		
29	Control	None	6,000,000, 18 hr. culture	" "		4920	7640	∞		70 hrs.	+	
30	Actively immune	12 injections, 1 cc. vaccine, intravenously	3,000,000, 18 hr. culture	Cistern		0	15	0		Lived		
31	Control	None	3,000,000, 18 hr. culture	"		27,000	∞			27 hrs.	+	+
32	Passively immune	10 cc. N.Y.S. serum intravenously	7,200,000, 18 hr. culture	"	0	60	700			48 "	+	++++
33	Passively immune	10 cc. N.Y.S. serum intravenously	4,800,000, 18 hr. culture	"	5	3640	2680			48 "	+	++++
34	Control	None	2,400,000, 18 hr. culture	"	1400	10,200	18,900			72 "	+	+
35	Normal	"	6,000,000, 18 hr. culture + fibrinogen	"	0	0	0			96 "	+	+
36	"	"	6,000,000, 18 hr. culture + fibrinogen	"	0	0	0			26 "	+	+++
37	"	"	6,000,000, 18 hr. culture	"	2400	∞	∞			22 "	+	+

injections each of heat-killed Type I pneumococci. Each injection consisted of the quantity of organisms contained in 1 cc. of 12 hour broth culture, centrifuged and resuspended to original volume in saline. Agglutination titers in these actively immunized rabbits did not exceed 1:5 after one series of injections, nor 1:20 after two series. Of about 80 animals examined, only one showed agglutinins in the spinal fluid. In a small series of rabbits actively immunized by both intravenous and intracisternal injections of killed Type I pneumococci, no agglutinins were present in the spinal fluids when examined 1 week after the final injection. It would appear, therefore, that any restraint observed in the progress of the pneumococcal meningitis locally in partially immune animals is not due to agglutinating antibodies in amounts capable of titration in the spinal fluid at the time of infection.

A considerable number of animals was immunized in the above manner. Subsequent infection was by direct inoculation of organisms in the cisterna magna. The preliminary experiments on restraining the septicemia were performed with an 18 hour broth culture, producing a growth of about 250,000,000 per cc. In all but the initial experiments the dosage was determined by the use of the Petroff-Hausser counter. Bleedings were made from the ear vein at varying intervals after infection, and the number of colonies per cc. of blood counted. A control non-immune rabbit was used for comparison for all doses of organisms. The results of immunization in controlling the development of the septicemic spread from the meninges are evident in Table I.

In all the experiments summarized in the first table, the organism used was type-specific and capsule-forming, and produced only the smooth variety of colony. In view of the recently developing interest in pneumococcus variants (R forms), it was considered desirable to test out such R forms for meningeal pathogenicity. The R form used was obtained from Dr. Avery and was derived experimentally from the same type-specific strain employed above. It was developed from a single cell colony.

Rough Type I pneumococci were injected intracisternally in eleven rabbits. Some were normal rabbits; others had recovered from *Streptococcus scarlatinæ* meningitis. In all, a transient bacteremia

TABLE II.

No.	Type	Dosage Type I R	Results of blood cultures				Duration of life	Autopsy cultures		Meningeal reaction
			1–1½ hrs.	3–3½ hrs.	5–5½ hrs.	24 hrs.		Heart	Cistern	
38	Normal	100,000,000, 18 hr. culture, cistern	1020	3760	800		24 hrs.	0	+	+
39	"	50,000,000, 18 hr. culture, cistern	300	35	25	0	Lived			
40	"	20,000,000, 18 hr. culture, cistern	5	15	5	0	"			
41	"	100,000,000, 18 hr. culture, cistern	0	160	525		24 hrs.	0	+	+
42	*Strep. scarlatinæ;* recovery	100,000,000, 18 hr. culture, cistern	5	5	5	0	Lived			
43	*Strep. scarlatinæ;* recovery	100,000,000, 18 hr. culture, cistern	0	30	0	0	48 hrs.	0	+ Very rare	+
44	*Strep. scarlatinæ;* recovery	250,000,000, 18 hr. culture, ear vein	3120	320	300	0	Lived			
45	*Strep. scarlatinæ;* recovery	100,000,000, 16 hr. culture, cistern	220	3640	35,280	0 (Paralysis)	14 days (Pneumonia)	0	0	0
46	Normal	100,000,000, 16 hr. culture, cistern	300	13,320	1740		24 hrs.	+	+	+
47	*Strep. scarlatinæ;* recovery	100,000,000, 16 hr. culture, cistern	960	440	225	0	7 days (Enteritis)	0	0	±
48	Normal	100,000,000, 16 hr. culture, cistern	240	215	65	0	78 hrs. (Pneumonia)	0	0	+

resulted (Table II). Only once, did the latter persist for 24 hours. Large doses of organisms were used, usually 100,000,000. This dose frequently killed. The mechanism of death is not clear. Few organisms could be detected in cistern fluid smears at death, but they were mostly phagocyted. The edema and congestion of the lungs were quite marked. If death did not occur within 24 hours, recovery was the rule. In a series of eleven animal passages, no reversion to a smooth colony type was observed.

It is obvious from consideration of Table I that active or passive immunization of animals previous to cisternal infection results in a retardation of septicemia. Where, exactly, this retardation occurs is difficult to determine on account of the multiplicity of factors involved, and on account of these variable factors, after a given degree of immunization the extent of the septicemia following intracisternal infection is irregular. The appearance of organisms in large numbers in spinal fluids is delayed. This retardation was especially notable in later experiments on treatment, where frequently, after an injection of upwards of 3,000,000 organisms intracisternally, the 19 hour cisternal fluid showed rare pneumococci only, and where in one instance spontaneous recovery of an untreated immune rabbit occurred, when all treated animals of that group died. It is likewise evident in early experiments (Table I), that in actively immune animals much larger infecting doses are required to produce a fatal meningitis. So far as was determined, this local meningeal retardation does not depend upon the presence of agglutinating antibodies in the fluid at the time of infection, although their ultimate influence is not beyond the realm of possibility, since following the cisternal injection, choroid plexus alteration may result in some diffusion of agglutinating antibodies present in the blood, or of opsonins (7). We have not done any opsonic studies on spinal fluids of immune animals.

In partially immune rabbits pneumococcal meningitis is occasionally abortive, but it would appear that in the meninges, doses of organisms will produce progressive disease and also eventual septicemia, whereas several times the number of diplococci injected intravenously will produce nothing at all. Therefore it may be assumed that the meningeal spaces constitute to some, and probably to a considerable extent, a non-immune locus, where organisms in numbers insufficient to

produce systemic disease may multiply, pass the filtration mechanism in great numbers, and break down the systemic resistance.

After the series of preliminary experiments outlined in Table I, we determined to try the effect of antisera in the control of the meningeal disease. Not much hope was entertained, in view of the severity of the process, but it was thought that if any evidence at all indicated benefit from serum therapy in so extremely susceptible an animal as the rabbit, there would be some justification in advocating its utilization. In the majority of instances, the concentrated antibody solution prepared for us by Dr. Lloyd Felton, of the Harvard Medical School, was used. According to Dr. Felton's titration, the samples employed by us contained 50 and 3000 protective units per cc. We must acknowledge that so far as beneficial therapeutic results are concerned, we were unable to see any difference in the two strengths of antibody solutions, nor could we detect that either differed appreciably from the Type I antipneumococcus serum prepared in the New York State laboratories. It is impossible to express results of therapeutic experiments in tabular form, and since it is not desirable to include all protocols, a short series only of the latter is included. It may be emphasized here that, whereas for convenience in planting and timing cultures, an 18 hour growth was used in preliminary studies of the septicemia, in therapeutic experiments a 6 hour culture was used. The first culture had obviously gone beyond its developmental peak, while the second was still in an active growth phase. It became apparent early that the second culture was much more active and that with given immunization a smaller dose of the 6 hour culture would duplicate the results of a larger 18 hour culture. It was likewise noted that the apparent vigor of the culture, as expressed by actual growth per cc. in a given number of hours, was also very important. Since the actual dosage of organisms in each instance was counted, we are forced to conclude that there must have been either an actual variability of potency in the individual cocci under unfavorable growth conditions, or possibly that the total constituency of the culture varied, cultures giving poor growth containing more damaged or dead cocci. These factors may be of minor importance in influencing ultimate fatal disease in normal rabbits, but may play a considerable rôle where partial immunity has been established. Then again, there

is an uncontrollable individual variation in animals; given a certain dosage of immune serum and a certain known cisternal dosage of pneumococci from the same culture, results will vary. These varying factors are shown in the following few representative protocols.

Illustrative Protocols.

Rabbit A.—Active immunity established by intravenous and intracisternal vaccine. Immunization completed December 4, 1926. December 14, 1926, 4.00 p.m., ether, cistern puncture, clear fluid; injected 17½ million Type I pneumococci. Good recovery. December 15, 9.30 a.m., temperature below 92°. Cistern puncture, rare cells but a massive, diffuse overgrowth of pneumococci. No phagocytosis. Injected 1 cc. Felton 50 unit antibody solution intracisternally, and 5 cc. antipneumococcus serum intravenously. Placed on electric pad. Temperature recovery to 100.2°. Dead 2.30 p.m. Autopsy: Brain is pale, meninges slightly opalescent. Cisternal fluid, instead of rare cells observed previous to treatment, contains, on smearing, from 60–100 polymorphonuclears per oil immersion field. Very active phagocytosis of pneumococci; free organisms largely agglutinated. Culture: massive growth; heart blood: no growth. Microscopically: fibrinopurulent exudate over cerebrum, cerebellum, medulla, and cord; exudate also involves dura, choroid plexuses, cord septa, sheaths of penetrating vessels. Large number of pneumococci. Phagocytosis confined essentially to region of cisterna. Moderate superficial encephalitis. Note: an example of massive proliferation of pneumococci in the meninges, no terminal septicemia, an initial poor cellular reaction in meninges, greatly improved by antibody injection, and induction of marked phagocytosis.

Rabbit B.—Same immunization and infecting dosage as in Rabbit A. Infected same day with same culture. 18 hours after infection, temperature 103.2°, sluggishness, fine tremors; 22 hours, hind limbs paralyzed. Cistern puncture under ether; cells 4–12 per o. i. f., moderate number of cocci, no phagocytosis. Injected 1 cc. 50 unit antibody, cisternally, and 5 cc. antiserum, intravenously. Temperature fell to 95°. 24 hours, second cistern puncture; cells 20–100 per o. i. f.; rare agglutinated cocci. First culture, massive growth; second, few colonies only. 42 hours: cisternal fluid contained large numbers of cocci. Injected 2 cc. antibody, with symptoms of pressure. 66 hours: fluid as above. Injected 1.5 cc. antibody. Death in 2 hours. Autopsy: Similar to Rabbit A, save that the lumbar cord is essentially gangrenous. Cisternal cell number again markedly increased (60–100 per field); agglutination and phagocytosis of cocci. In addition to usual microscopic findings, considerable periarteritis of larger vessels of cord meninges, purulent ventriculitis of third and lateral ventricles, abscesses of choroid plexuses of fourth and lateral ventricles, and ingrowth of cocci into wall of lateral ventricle. Summary: Same immunization and dosage, but a considerably lesser initial process. Repeated treatments always

resulting in increased cellular reaction and phagocytosis, but in the interim between treatments great augmentation of cocci from regions untouched by treatment. Heart blood: no growth.

Rabbit C.—Immunization as in the two preceding. December 16, 1926, 4.00 p.m., ether, cistern puncture, clear fluid. Injected 12 million Type I pneumococci, 7 hour culture. 18 hours later, temperature 106.5°. Cisternal fluid opalescent, cells 10–20 per o. i. f.; only occasional clusters of diplococci; culture slight growth. By vital staining cells 64 per cent polymorphonuclears, 34 per cent monocytes, 2 per cent small lymphocytes. Injected 1.5 cc. antibody solution. 23 hours, lumbar and cistern punctures; leucocytes increased; very few phagocytic cocci; none free. Cultures: no growth. Injected 1 cc. antibody in cistern and in lumbar cord. No further treatment and an uneventful recovery. Summary: Delayed development of meningeal infection with few organisms in fluid at time of treatment. Rapid sterilization of a mild process. Recovery not necessarily due to treatment since immunization may have played the more decisive rôle.

Second animal of this group, similarly treated, developed a massive growth of cocci and died in 41 hours with only moderate meningeal reaction and a slight septicemia. These two rabbits emphasize the individual factor. A control, actively immune, untreated rabbit died in 41 hours with severe septicemia and a good meningeal reaction.

Rabbit D.—December 13, 1926, 9.50 a.m., 10 cc. Type I antipneumococcus serum, ear vein. 4.10 p.m., ether, cistern puncture, clear fluid, injected 3,000,000 Type I pneumococci, 7 hour culture. 18 hours after infection temperature 106.4°. Cisternal puncture; fluid slightly opalescent; cells very rare and only occasional cocci. Culture slight growth. Injected 1 cc. 50 unit antibody. 24 hours after infection second cistern puncture; fluid abundant, almost purulent; polymorphonuclears about 100 per o. i. f.; no cocci seen; culture negative; 1 cc. antibody injected. 42 hours, temperature 105.4°; active. 66 hours: appears slightly ataxic and has a head nystagmus. Spinal fluid culture negative. 5 days: temperature 104.5°; improved. 7 days: unchanged. 10 days: found dead. Autopsy: Spinal cord and brain pale, meninges opalescent. Cisternal fluid seropurulent, containing 50–100 polymorphonuclears per o. i. f. and a diffuse overgrowth of pneumococci. Marked congestion and edema of lungs. Culture of heart blood, no growth. Usual histology of fibrinopurulent meningitis. Summary: Low grade initial process in passively immune rabbit; culture of cisternal fluid twice negative after antibody injections. Final recrudescence of meningeal infection leading to delayed death at 10 days.

A second rabbit of this group, similarly immunized and infected, developed a massive growth of pneumococci within the first 18 hours, with essentially no cellular reaction. The latter was greatly improved by 1 cc. of antibody and at death, 3 hours later, there were large numbers of leucocytes and phagocytosis of 50 per cent of the organisms in the cisternal fluid. Blood cultures gave moderate growth.

The passively immune, untreated control died of meningitis and septicemia in 36 hours.

Rabbit E.—December 20, 1926, 10.00 a.m., 10 cc. Type I antipneumococcus serum ear vein; 4.00 p.m., 3,000,000 Type I pneumococci intracisternally. 18 hours: temperature 106.2°; active. 42 hours: temperature 106.3°; sluggish; diagnostic cistern puncture; cells rare; pneumococci very rare; culture positive; blood sterile. 66 hours: temperature 105.4°; sluggish; marked ataxia and weakness; diagnostic cistern puncture; essentially no change in fluid picture. 88 hours: dead. Petechial hemorrhages over cerebellar hemispheres; meninges opalescent, brownish, gelatinous looking exudate at base; cord not grossly remarkable; cisternal fluid smear shows about 100 leucocytes per o. i. f. and a large diffuse overgrowth of diplococci. Practically no phagocytosis. Microscopically: a thick fibrinopurulent meningitis. Summary: A markedly delayed initial process with essentially no early cellular reaction; a terminal extensive leucocytosis with little phagocytosis and a diffuse coccal overgrowth.

Of this group two animals were treated; both had mild initial processes at 18 hours and were lavaged from lumbar to cistern with antibody solution. One died at 88 hours with a pneumonia from which Type I pneumococci were isolated; at death both spinal fluid and heart blood gave no growth. The other animal recovered. Although this effect would appear to indicate favorable results of treatment, in our opinion it means nothing. There are too many variable factors involved to make correct analysis possible.

In recovered rabbits, various postmeningitic signs may occur. Prominent among these are paraplegias, ataxia, nystagmus, and prolonged opisthotonus. With the exception of the paraplegias, there is a strong tendency to amelioration of these conditions.

Whereas in small series of three to five rabbits, similarly immunized and infected, controls may die and treated animals recover, in one instance the reverse has been true, four treated animals dying, and one untreated recovering. This occurrence only goes to prove the impossibility of evaluating results when such variable factors are present. Individuals react differently after the same immunization; there is a very narrow zone between a dose of organisms which in a partially immune animal may produce a massive growth of cocci in the meninges with or without septicemia, and a dose which may permit spontaneous recovery. If we take length of life as an index of value of therapy, it is apparent that the treated animals dying, lived an average of 24 hours longer than the controls; if we consider the presence or absence of septicemia, it becomes evident that 28 per cent of animals treated locally and systemically subsequent to infection had positive blood cultures at autopsy and that 70 per cent of partial immunes with no subsequent treatment had terminal septicemia. Length of life in treated animals is difficult to interpret on account of a high percentage of traumata with repeated punctures; working space is very small in the rabbit, and the needle permitting satisfactory lavage is of course disproportionately large. Reac-

tion to intracisternal injection of antibody solution is often pronounced. We may summarize it in the following protocol.

Rabbit F.—Actively immune; uninfected; temperature 102.5°. Ether; cistern and lumbar puncture; clear fluid. Lavage with 2 cc. antibody solution, strength 50 units per cc. Immediate ether recovery. Within 10 minutes, prostration, tremors, chill, progression movements of all four limbs, rapid, shallow respiration, temperature 95.7°, falling in 2 hours to 92°. Following morning slightly sluggish, temperature 99.5°. Subsequent uneventful recovery.

In one instance the reaction did not begin until 1½ hours after injection of antibody. Samples of antibody containing no tricresol produced at times similar effects. These reactions may be duplicated by trauma to medulla, but frequently no trauma could be verified at autopsy. In the uninfected animal intrathecal antibody solution causes a slight transient polymorphonuclear reaction, rapidly passing over to an exudate containing small lymphocytes and endothelial leucocytes.

Pathology.

The microscopic appearances of the pneumococcal meningitis have been studied in all the animals. The pathology is distinctly a function of immunization, dosage, and treatment. At one extreme one has the non-immune rabbit infected *via* the cisterna. The tendency in this animal is for the cerebrospinal fluid to be turned into a veritable culture medium for pneumococcus (Fig. 3); the pia-arachnoid is filled with a massive sheet of pneumococci; the choroid plexuses contain them in great numbers; they invade the Virchow-Robin spaces in solid masses (Fig. 4); they multiply within the ventricles. In this form of disease there is very little reactional change in the meninges; death is always with marked septicemia usually complicated by edema and congestion of the lungs. In a partially immunized rabbit death is delayed, and time is allowed for a vigorous cellular reaction in the meninges; this takes the form of a thick, fibrinopurulent exudate, involving the meninges of all regions, cord, cerebellum, convexity, base and choroid plexuses. Its intensity is variable. Pneumococcal proliferation may be restrained at first, but eventually usually reaches an extreme degree, the cocci lying in solid masses between reacting cells (Fig. 5); there is little phagocytosis. If there is intrathecal treatment, an increased leucocytosis, with active phagocytosis, agglutination, and thread reaction result (Figs. 6–8). Phagocyted cocci stain supravitally with neutral red. These phenomena are but transitory and

within a few hours there is a tendency for cells to diminish and for the diffuse growth of organisms to be resumed. This constant renewal of pneumococci originates from agglutinated, unphagocytized clusters of pneumococci and particularly from regions untouched by treatment. It is evident that it is impossible to reach, by treatment, all regions in the rabbit, if the initial growth of bacteria is at all severe. Exudate with pneumococci may be found in the central canal of the cord, between fibers of issuing nerve trunks (Figs. 9 and 10), or in spinal ganglia (Fig. 11). There may be empyema of the central canal of the cord, with destruction of the ependyma and involvement of either the cornua or funiculi (Fig. 12). Pneumococci in the sheaths of penetrating vessels are commonly observed. In any severe process, one is apt to find a superficial encephalitis (Figs. 13 and 14), or else foci of softening and invasion by pneumococci (Fig. 15). Periarteritis is common (Fig. 16). Purpuric hemorrhages may occur either in the meninges or deep in the brain or cord substance (Fig. 17). Death is almost always complicated by edema and congestion of the lungs. There may be a considerable fibrin deposit and if life is sufficiently prolonged, a lobular consolidation may result (Figs. 18–20). Pneumococci may be demonstrated in this exudate by Gram-Weigert staining.

SUMMARY AND CONCLUSIONS.

1. Fatal Type I pneumococcal meningitis may be produced in rabbits by intracisternal injection of pneumococci.

2. When organisms are of high virulence, the rabbit does not tend to localize them in the meninges, but an early septicemic process results. Death is septicemic rather than meningeal.

3. In such instances very little cellular reaction occurs in the meninges.

4. Active or passive immunization previous to intracisternal infection inhibits partially the septicemia and permits the development of reactional processes in the meninges.

5. The immunization likewise retards the meningeal disease, but multiplicity of factors prevents us from stating precisely to what this retardation is due. It is not correlated with the presence of agglutinins in the spinal fluid at the time of infection.

6. The rapidity of production of meningitis is influenced by the

phase of growth of the culture used, and likewise by the growth activity of that culture.

7. To some extent in the partially immune rabbit the meningeal spaces constitute a relatively non-immune reservoir, constantly feeding the blood stream and breaking down systemic resistance.

8. Intrathecal serum treatment causes rapid agglutination and phagocytosis of pneumococci, and has very rarely, possibly, resulted in cure. Essentially no phagocytosis occurs in the absence of immune serum.

9. Phagocyted pneumococci stained supravitally take up the neutral red stain and are therefore probably injured.

10. The treatment employed subsequent to infection only slightly prolongs life in the majority of cases. It does retard septicemia.

11. The treatment improves the cellular reactional processes in the meninges.

12. A study of the pathology of rabbit pneumococcal meningitis shows that the location of pneumococci precludes complete contact with serum introduced intrathecally, and that these locations provide isolated foci, from which organisms may reinfect the meningeal spaces as rapidly as they are removed by lavage or antibody injections.

13. In recovered rabbits postmeningeal symptoms, weakness, ataxia, nystagmus, and paralyses arise.

14. In our opinion there is some objective evidence of benefit of serum therapy. The rabbit is too susceptible, however, and conditions too artificial to admit of definite conclusions.

15. Rough Type I pneumococci introduced in large quantity cisternally may kill and may be recovered 24 hours after infection, from spinal fluids. In a series of eleven passages, no reversion to smooth type occurred. In all animals injected with rough forms, transient bacteremia resulted.

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EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Non-immune rabbit. Pneumococci in lung capillaries. Pulmonary edema. Gram-Weigert stain. × 1000.

FIG. 2. Same animal, stain, and magnification. Pneumococci in capillary of renal medulla.

FIG. 3. Spinal fluid smear. Shows type of fluid found in a non-immune rabbit or in one partially immune, which has developed a massive overgrowth of cocci previous to treatment. × 1000.

FIG. 4. Non-immune rabbit. Solid masses of pneumococci in perivascular sheath of penetrating cortical vessel. Gram-Weigert stain. × 1000.

PLATE 12.

FIG. 5. Massive growth of pneumococci in partially immune treated rabbit. Slight phagocytosis. Section of cord far distant from region of treatment. Gram-Weigert stain. × 1000.

FIG. 6. Agglutination and vigorous phagocytosis in exudate of treated animal. × 1000.

FIG. 7. Thread reaction in agglutinated cocci after treatment. × 1000.

FIG. 8. Phagocytosis of pneumococci in exudate of treated animal. The same animal as in Fig. 5, but from region near location of treatment. Gram-Weigert stain. × 1000.

PLATE 13.

FIG. 9. Polymorphonuclear invasion of spinal nerve trunk. × 260.

FIG. 10. Growth of pneumococci between fibers of spinal nerve trunk. Non-immune rabbit. Gram-Weigert stain. × 1000.

FIG. 11. The same animal, stain, and magnification. Pneumococci in a spinal ganglion.

FIG. 12. Cord abscess (pneumococcal) in a partially immune rabbit; an extension from empyema of central canal of cord. × 150.

PLATE 14.

FIGS. 13 and 14. Superficial encephalitis. Partially immune rabbit. × 260.

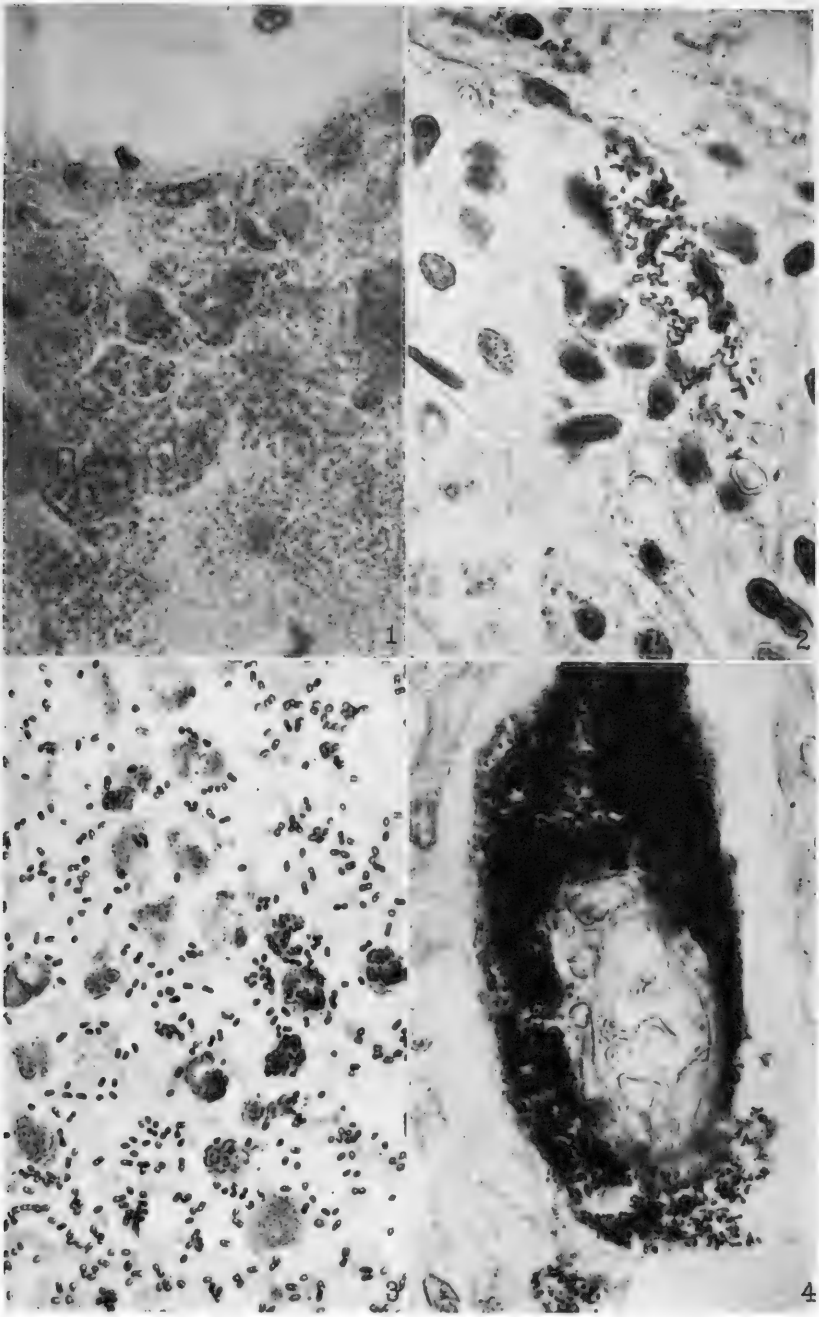
FIG. 15. Pneumococcal invasion with softening of wall of lateral ventricle. Treated animal dying at 21 hours. Gram-Weigert stain. × 1000.

FIG. 16. Periarteritis, meningeal cord vessel. Partially immune rabbit. × 260.

PLATE 15.

FIG. 17. Hemorrhagic lesion; gray matter of cord. Partially immune rabbit dead 28 hours after infection. × 110.

FIGS. 18 to 20. Pneumococcal pulmonary lesions as observed in partially immune rabbits. × 50.



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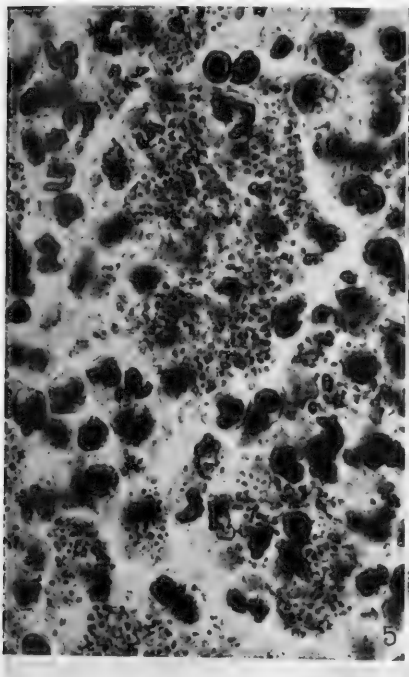

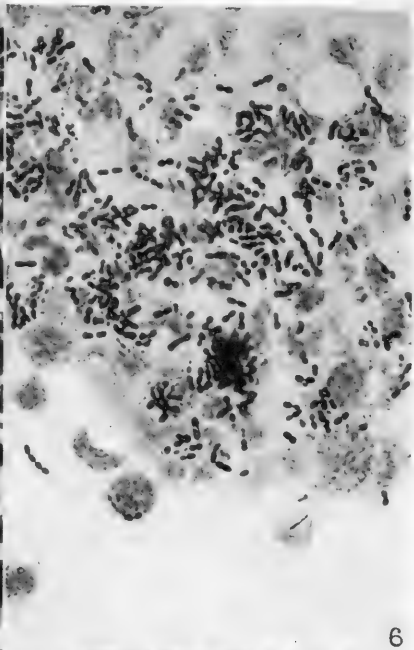

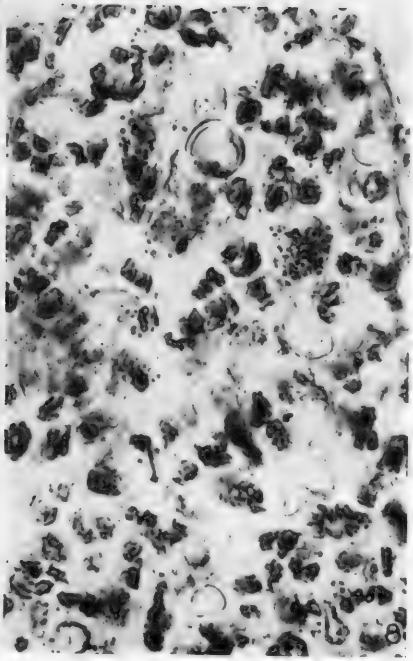

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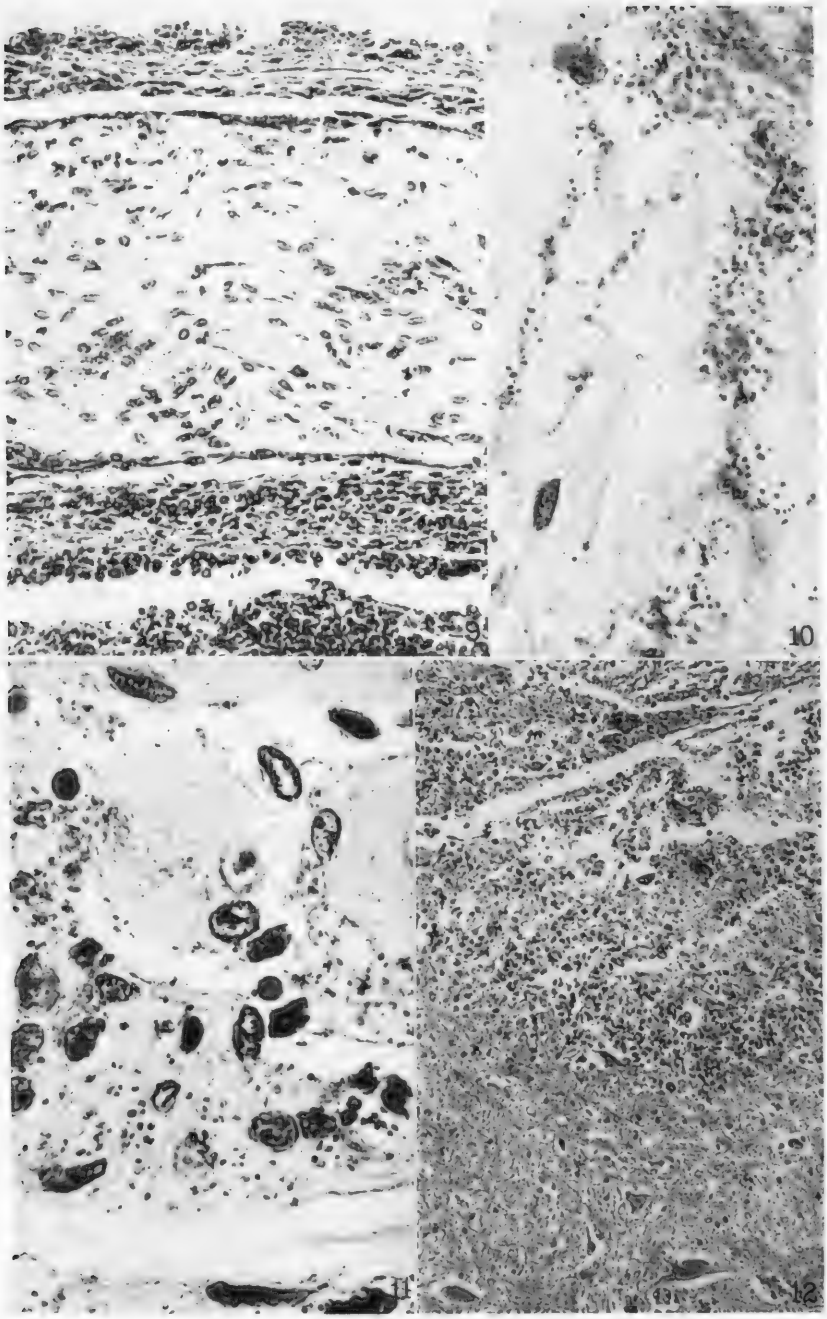

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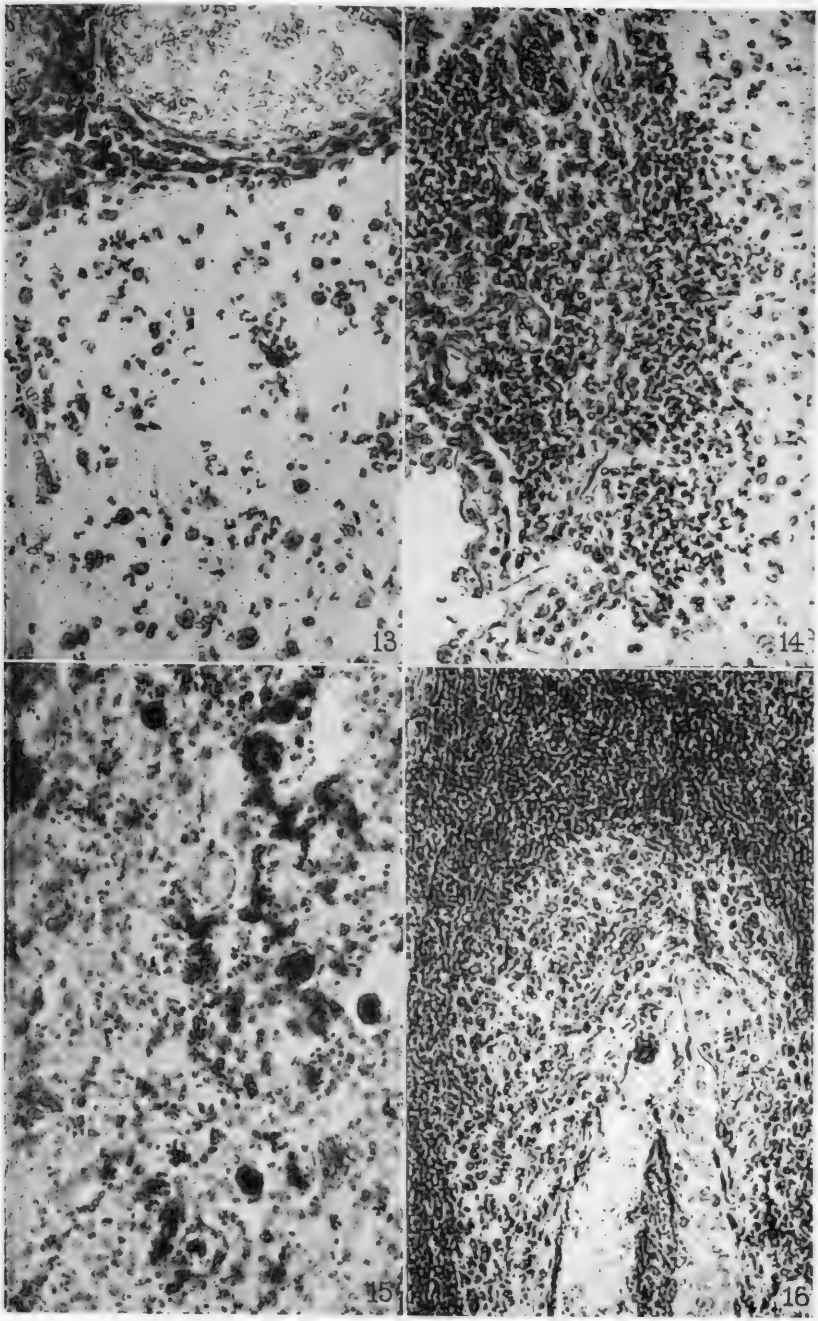

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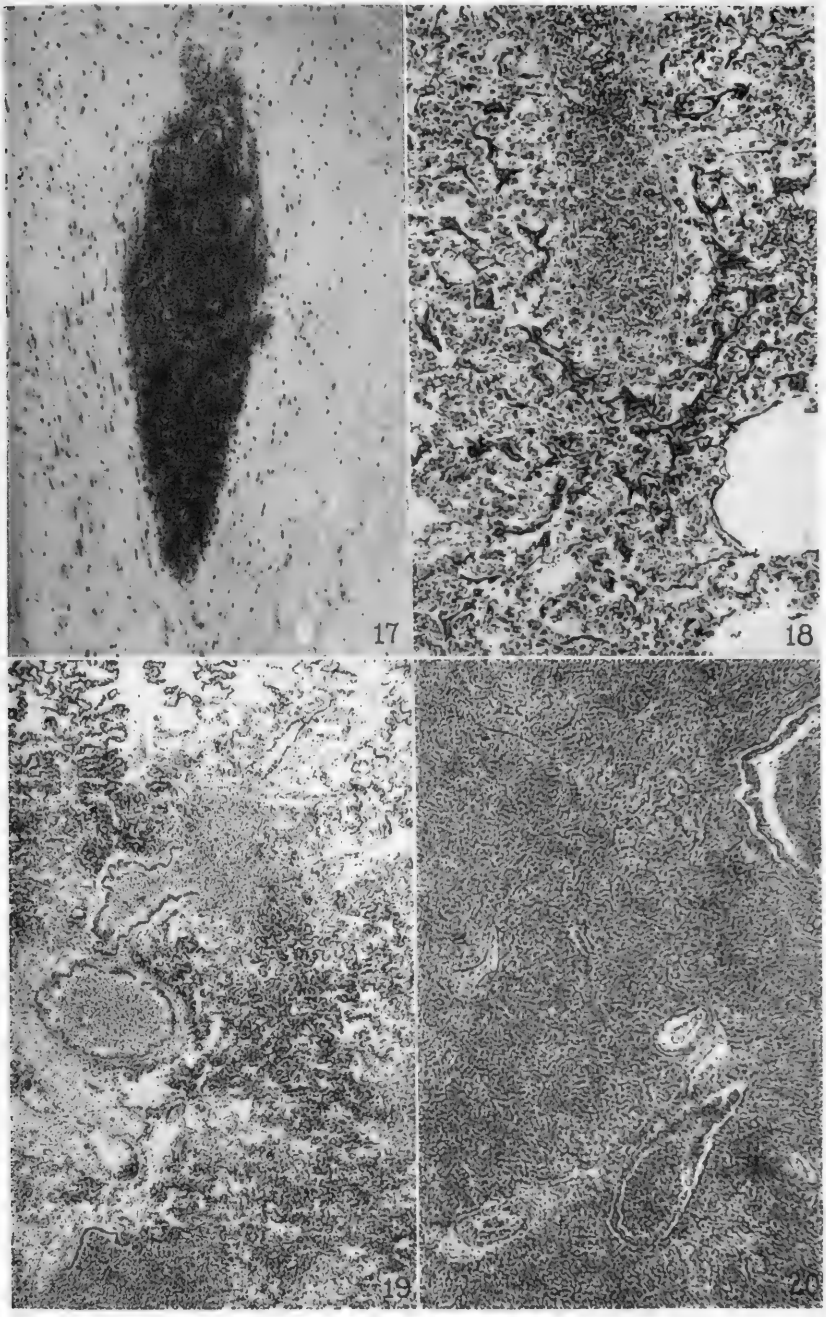

(Stewart: Pneumococcal meningitis. I.)

LOCAL SPECIFIC THERAPY OF EXPERIMENTAL PNEUMOCOCCAL MENINGITIS.

II. The Production, Pathology, and Treatment of Type I Pneumococcal Meningitis in Dogs.

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Plates 16 to 21.

(Received for publication, June 12, 1927.)

In the previous paper (1) the production, pathology, and attempted treatment of Type I pneumococcal meningitis of rabbits were discussed. Reasons were presented explaining why the rabbit is a very unfavorable animal for the study of this disease, which may be briefly summarized as follows: In a normal rabbit, septicemia, following intracisternal infection with Type I pneumococci, is invariable, rapid, and massive, and septicemic death occurs before there is any reactional localization in the meninges; consequently, to produce meningeal localization, complete or partial, a previous immunizing treatment is necessary. Following this treatment, however, there is a considerable variation in the intensity of the meningeal infection, making it impossible to gauge properly the dosage of organisms which will produce fatal disease, but which will not immediately overwhelm the animal's resistance. Even if the disease intensity is properly regulated, treatment is unsuccessful on account of both the limited working space and the widespread localization of organisms in regions impossible to reach either by immune serum or lavage.

In view of these facts, the rabbit was abandoned as an experimental animal and recourse was had to young dogs. The dog is, as we know, relatively resistant to systemic pneumococcal infection, although marked variations even here are encountered. In the meninges, however, a type of disease may be produced which progresses toward fatal termination, if cultures of suitable virulence are used, and one which quite closely approximates pathologically the human pneumococcal

meningitis. To produce rapid progressive meningitis with any degree of assurance that the disease will assume a fairly characteristic course from day to day, the infecting dose must be reasonably standardized. In the present study the dosage of organisms has been accurately counted by means of a Petroff-Hausser bacterial counter. Not only should the numbers of infecting pneumococci be known, but the culture should be standardized both as to hours of growth and as to quantity of growth. A culture should be chosen which is well along on the upward limb of the growth curve. We have employed 6 hour cultures. It must be a culture which has given a good growth. There is a marked difference between the degree of 18–24 hour infection produced by a given number of pneumococci from a 6 hour plain broth culture which has produced a low total growth, *i. e.*, a growth ranging from 150–225 million per cc., and that produced by the same number of organisms from a 6 hour 0.2 per cent dextrose broth culture which is producing a growth of upwards of 700 million per cc. Unless these differences are recognized, conclusions are of little value. Use of late (24 hour) cultures may lead to very deceptive results. Even with these cultural conditions standardized, there is an uncontrollable individual factor which influences the rate of establishment of progressive disease; every animal is an individual and must be so handled. Whereas we hesitate to interpret the anatomic and therapeutic results of others, we are inclined to believe that the findings of Idzumi (2) were the result of the use of a culture of low virulence. Idzumi studied pneumococcal meningitis in dogs. This investigator used enormous doses of organisms, from 1–2 cc. of a suspension secured by centrifuging 10 cc. of a 24 hour culture of low mouse virulence. Although the dogs died, the pathologic changes were scarcely more than those of hyperemia. In our own study the disease has been regularly produced with approximately 1 cc. of a 1:100 dilution of 6 hour culture. It would seem that Kolmer's (3) successful therapy, *i.e.*, lavage from one or both lateral ventricles to the cisterna magna with physiological saline or Ringer's solution, cannot be interpreted as the result of other than feeble infection with cultures well beyond the growth peak and full of non-viable organisms. The lavage was most incomplete, since the entire cord, base, and convexity were untouched by the saline. Kolmer reports, however, that at the time of lavage

the spinal fluids were always purulent and contained myriads of pneumococci, making it more difficult for one to understand how lavage from *one* lateral ventricle to the cisterna magna could have appreciably influenced the process, since in our own experience the other ventricle would always have been an untouched focus in a disease of the intensity he describes. Bull (4) observed meningitis developing in the course of pneumococcal septicemia in dogs. His organism was not typed. Bull noted some spontaneous recoveries.

EXPERIMENTAL.

Apparently in control dogs, infected intracisternally, death may occur in one or more of three ways. A dog may develop a fairly rapid proliferation of pneumococci in the meninges with a constant large feeding of organisms into the blood stream, and die of bacteremia. If a culture is of low potency due to poor growth conditions, a meningeal process of such intensity may result that a very marked cellular reactional process occurs, with pneumococci present in relatively small numbers. In such cases death appears to be "reactional." Thirdly, the proliferation of organisms within the meninges may attain enormous proportions, and brain and cord may be covered with a thick, gelatinous layer, consisting mainly of heavily encapsulated pneumococci, but with relatively moderate cellular reaction. In the second and third instances, death may be due, in part at least, to the mechanical obstruction of the meningeal spaces. The actual mechanism of death from pneumococcus infection is always rather obscure. Three protocols may be introduced to illustrate these points.

Dog 12.—Female hound; weight 5.5 kilos. March 23, 1927, 4.10 p.m., morphine gr. 1/5; ether; cistern puncture; clear fluid. Injected 6,500,000 Type I pneumococci (6 hour dextrose broth culture, growth 1,100,000,000 per cc.). Good recovery. 19 hours later, lethargic, weak, irritable, sicker than usual at this stage. 23 hours, morphine gr. 1/5; death in 10 minutes. Autopsy: Brain and cord injected, edematous; cisternal fluid cloudy; marked grayish yellow opacity along sulci of convexity and at base. Smears from the usual regions show on an average 20–30 pneumococci per oil immersion field and 30–60 cells, mostly polymorphonuclears. Viscera negative. Culture from heart's blood gave massive confluent growth. A death mainly due to septicemia. Microscopically, however, there was a marked diffuse fibrinopurulent leptomeningitis.

Dog 3.—Female hound; weight 7.5 kilos; February 9, 1927, 4.20 p.m., ether,

cistern puncture, clear fluid. Injected 15,000,000 Type I pneumococci (7 hour plain broth culture giving poor growth). Good recovery.

23 hours—temperature 103°. Sluggish; refuses food.

43 hours—temperature 100.2°. Unchanged; 100 cc. saline intraperitoneally.

67 hours—temperature 103°. Sleeps most of time; able to stand; 75 cc. saline intraperitoneally.

93 hours—temperature 102.2°. Able to stand leaning against cage; weak; stiffness of neck; 75 cc. saline.

5th day—temperature 102°. Very irritable.

6th day—temperature 103.2°. Irritable; chills; tremors.

7th day—temperature 99.8°. Prostrate; hypersensitive; tremors; incontinence of urine and feces; 100 cc. intraperitoneal saline.

8th day—dead. Autopsy: Upon opening spinal cord and cranial cavity, meninges are opaque and pinkish yellow; purpuric blotches in region of medulla. Cord dura is very tense; on slitting it, thick, grayish or greenish yellow pus exudes; this is generalized in distribution, but most abundant in lower medullary, upper cervical, and region of lumbar enlargement. Smears show thick masses of polymorphonuclear leucocytes and rare endothelials. Only occasional diplococci, all extracellular. Over the cerebellar vermis and adjacent portions of cerebellar hemispheres is thick pus. A smear shows a similar picture to that from cord. The base of the medulla and the pons is coated with thick, greenish yellow pus (Fig. 1). Much less exudate over convexity, and pneumococci are rare. The lateral ventricles are dilated and filled with thick pus, and the walls appear eroded. In the ventricular pus cocci are more numerous, but still do not reach the usual proportions; are free and in small agglutinated clusters. Essentially no phagocytosis. Heart's blood cultures are negative.

Summary: Infection with culture giving poor growth; delayed death; enormous reactive process; few pneumococci at autopsy. Suggests a "reactional" death. Histologically a very marked meningitis, superficial encephalitis and myelitis.

Dog 7.—Male fox terrier; weight 6 kilos. March 9, 1927, 4.05 p.m., ether; cistern puncture; clear fluid. Injected 7,000,000 Type I pneumococci (6 hour dextrose broth culture, growth 700 million per cc.). Good recovery.

18 hours—temperature 103.5°. Stands, wags tail, refuses food and water.

42 hours—temperature 103.4°. Semiprostrate; 100 cc. intraperitoneal saline.

66 hours—very irritable; unable to stand.

90 hours—dead. Autopsy: Fourth ventricle distended with thick, gelatinous material; cord meninges and meninges over convexity and base tense, opaque, filled with similar yellowish gelatinous deposit. Lateral ventricles contain thick pus. Smears from all regions show numerous pus cells, but an incredible number of pneumococci (Fig. 2), the latter really making up most of the gelatinous material. Essentially no phagocytosis. Both cerebrospinal fluid and heart's blood give abundant growth.

At this point, the observation should be emphasized that smears taken with a loop from different regions of brain or cord meninges give rather deceptive results so far as numbers of exudative cells are concerned. Probably this is due to the fact that cells lie in fibrin meshes wherein they are held. Pneumococci may be picked up more easily and smears, so far as numbers of bacteria go, check fairly well with microscopic findings in sections.

Therapeutic Experiments.

After a few trials of the culture to determine a proper infecting dose, attempts were instituted to treat the animals. The first series consisted of three dogs, two treated and one control. The culture used was that described in the protocol of Dog 3 (above). This culture was of low virulence, producing a prolonged process in the control animal. The infecting dose consisted in 2,000,000 organisms per kilo of body weight; animals were injected intracisternally. It so happened that both treated dogs made uneventful recoveries, save that one had a residual deafness. These animals were treated at an early period in the work, really before we had had much experience in puncturing the cistern and lumbar subarachnoid space, and the daily progress of the disease was not sufficiently followed, nor was the condition of the fluid of one of the treated animals determined previous to treatment. The fluid of the other animal showed but 4–6 cells per oil immersion field, and but 4 diplococci in the entire smear; the culture was nevertheless positive. Treatments were in one instance with Felton's 3000 unit antibody solution, and in the other with unconcentrated antipneumococcus serum. The details of these treatments are not included, since from the behavior of the culture and of the control animal, we feel inclined to disregard this experiment. Suffice it to say that no subsequent test with really virulent culture has promised any duplication of this favorable result. The following protocol summarizes a more systematic study of the effect of treatment, and is introduced to show the behavior of a treated dog for which Dog 7, the animal described above in illustration of the massive pneumococcal overgrowth type of disease, was the control.

Dog 6.—Female bull terrier; weight 5 kilos. March 9, 1927, 3.50 p.m., ether; cistern puncture; clear fluid. Injected 7,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 700 million per cc.). Good recovery.

18 hours—temperature 104.7°. Stands, wags tail, refuses food and water.

19 hours—ether; cistern puncture; lumbar puncture; lavage from lumbar to cistern with 20 cc. warm saline, followed by 15 cc. antipneumococcus serum; 5 cc. 800 unit antibody solution intravenously. Cisternal fluid opalescent; looks like a suspension of cocci. Smears of this fluid as follows:

Before lavage—cells 20–30 per oil immersion field; myriads of cocci.

After 15 cc. NaCl lavage—cells rare; cocci about 80 per o. i. f.

After 20 cc. NaCl lavage—cells rare; cocci about 60 per o. i. f.

24 hours—temperature 103°; ether; cistern puncture; seropurulent fluid; drainage. Injected 5 cc. antipneumococcus serum. Smear: numerous pus cells; cocci largely agglutinated and phagocyted; still numerous, but less than at the end of the previous lavage. 75 cc. intraperitoneal saline. Culture: confluent growth.

42 hours—temperature 101.6°. Weak, sluggish, able to stand. Ether, combined puncture; cloudy yellowish fluid; 20 cc. saline lavage, followed by 10 cc. antipneumococcus serum. Smear: before lavage, polymorphonuclears 30–150 per o. i. f.; cocci not increased over previous smear. After completing lavage, both cells and organisms very rare. Culture: confluent growth.

66 hours—temperature 101.7°. Refuses to stand; lethargic; shortly after coming to laboratory a generalized convulsion with salivation and gnashing. Series of bloody stools. Ether; combined puncture; cells 3–60 per oil immersion field; cocci diminished; many look swollen; bacillary; coccoid. 10 cc. antipneumococcus serum.

90 hours—dead. Autopsy: Cord grossly normal; vessels in region of cerebellum and medulla injected; posterior convexity injected; fibrinopurulent material over anterior convexity and base. Thin seropurulent material in lateral ventricles. Smears: cistern—cells 125 per o. i. f.; cocci largely phagocyted. Cord—cells 50 per o. i. f.; cocci very scanty. Anterior convexity—cells 200 per o. i. f.; cocci diffuse with only slight phagocytosis. Lateral ventricles—cells 30–40 per o. i. f.; cocci diffuse with marked phagocytosis. Heart's blood culture—no growth. Viscera: acute intussusception (the cause of death). Microscopically the cord showed relatively little exudate, but the latter was very abundant over the convexity and cerebellum and in the fourth ventricle.

Summary: A very marked initial growth of cocci; clearing by repeated lavage; abundant phagocytosis of residual cocci following antiserum injections but a focus, especially over the anterior convexity, not appreciably reached by serum and thereby constituting a site for subsequent "reinfection" of other regions. Objectively a very decided benefit from treatment.

In view of the fact that it appeared quite possible to lavage certain regions of the central nervous system relatively free from organisms

and to reduce residual penumococci by the phagocytosis resulting from the injection of immune serum, it was considered desirable to extend, if possible, the field of action of the serum to those portions of the brain not reached by lumbar or cisternal injections. It was determined to treat the convexity by subdural serum injections, after trephining over the frontal lobes. The question arose as to whether one trephine was sufficient, or whether the falx constituted such a complete barrier that bilateral trephining was necessary. To settle this point a single frontal trephine was made just above the frontal sinus in a normal dog under ether anesthesia and 2 cc. of methylene blue was injected. The animal later received likewise 2 cc. intracisternally. The methylene blue injected frontally passed rapidly to the cisterna and with the additional cisternal injection was almost immediately recovered from the lumbar subarachnoid space. The dog was etherized after 1 hour. Staining of the brain was, as far as the convexity was concerned, sharply limited to the hemisphere of the injected side (Fig. 3). This made it obvious that to treat the infected convexity bilateral trephine openings were necessary. The following protocol summarizes one of the early animals treated by quadruple puncture.

Dog 10.—Fox terrier, female; weight 7.5 kilos. March 18, 1927, 3.15 p.m., ether; cistern puncture; clear fluid. Injected 8,400,000 Type I pneumococci (6 hour dextrose broth culture; growth 1,400,000,000 per cc.). Good recovery.

21 hours—temperature 99.8°. Sluggish; refuses to stand; will not drink. Ether; lumbar puncture; cells 3–5 per o. i. f.; no cocci seen, but culture positive. Cistern puncture; cells 15–25 per o. i. f.; rare cocci; culture, marked growth but not confluent. Lavage lumbar to cistern with 15 cc. saline followed by 20 cc. antipneumococcus serum. Cistern needle withdrawn and left frontal trephine done. Injected very slowly 6 cc. serum. In view of the few organisms in the smear, it was felt that a second frontal trephine was not necessary.

45 hours—temperature 103.6°; unchanged clinically. No treatment.

66 hours—temperature 103.7°; clinically unchanged.

70 hours—diagnostic lumbar puncture; cells 3–5 per o. i. f.; cocci numerous.

72 hours—cistern and lumbar punctures; lavage of 15 cc. saline lumbar to cistern; followed by 15 cc. serum. First cisternal smear shows several hundred pneumococci per field; smear following treatment shows diminution and agglutination of cocci. Animal left head down for 30 minutes.

90 hours—temperature 102.7°; irritable but otherwise not notably worse. Cistern and lumbar tap. Cisternal fluid, yellow, purulent; contains about 150 cells per field; cocci markedly decreased and phagocyted. Culture: confluent growth. Lavage lumbar to cistern with 15 cc. serum. Rapid ether recovery.

114 hours—temperature 100.9°; weakness of hind legs; irritable; cistern and lumbar punctures; fluid nearly clear. Second trephine done.

Smears: lumbar—rare cells; no organisms seen. Cistern—rare cells; rare agglutinated pneumococci.

Cultures: fair growth.

Injected 2 cc. serum *via* each frontal trephine. 6 cc. lumbar subarachnoid space. 4 cc. cisterna.

Since sterilization was incomplete after several treatments, it was decided to test out the efficacy of ethylhydrocupreine hydrochloride (optochin) intrathecally. This effect is apparent in the continued protocol.

138 hours—temperature 99.4°. Unable to stand; lethargic. Lumbar and cistern punctures under ether. Cisternal smear: cells 5–10 per o. i. f.; cocci 15–20. Lavage with 15 cc., 0.0002 per cent ethylhydrocupreine hydrochloride in saline. Normal recovery.

168 hours—temperature 102°. Lumbar and cistern punctures under ether; cisternal fluid purulent; cocci very numerous. Injected 6 cc. 0.02 per cent optochin.

186 hours—usual double puncture; fluid contains hundreds of pus cells per field; cocci are diminished and markedly phagocyted. Injected 8 cc. of mixture of 15 cc. 0.2 per cent optochin and 5 cc. serum. Breathing ceased but was immediately renewed upon giving artificial respiration. Culture: rare colonies.

210 hours—dead. Autopsy: Moderate exudate over cord; brain congested; easily broken, fibrinous adhesions about cistern and over convexity; moderate seropurulent exudate at base and in both lateral ventricles; the latter are considerably dilated (Fig. 4). A small puncture wound in the floor of the fourth ventricle, just above calamus, with small hemorrhage extending into the central canal of the cord; this was the probable cause of death and is the inevitable result of frequent successive punctures into a dangerous region where working space is small. Smears from all regions show pneumococci—scanty in the cord, moderate in cistern and over convexity, fairly abundant at base and in lateral ventricles. The cellular reaction parallels the pneumococcus distribution in intensity. The principal feature in all smears is the extraordinary amount of phagocytosis and destruction of pneumococci. Many cells are loaded with bacterial débris (Fig. 5). Cultures: all regions positive; heart blood: no growth.

Summary: A mild initial infection; first treatment incomplete; considerable delay in instituting subsequent treatments; the latter usually not complete. Finally recourse to optochin in low concentration, resulting in exacerbation in growth of organisms. Gradual tendency toward sterilization with higher drug concentrations; course interrupted by traumatic death. A total of seven treatments, usually only partial in distribution.

As will be seen from the protocol, the initial growth of organisms in the spinal fluid was described as mild. We have made certain observations as to the rate of increase of these mild initial growths; for example, the fluid in one dog infected with $4\frac{1}{2}$ million pneumococci is described as follows: 19 hours, 7 diplococci found in the entire smear; 23 hours, an average of 10 per oil immersion field; 42 hours, upwards of 100 cocci per field. The speed of progress of the infection becomes very evident and the significance of even a delay of a few hours in instituting treatment is obvious.

One dog afforded opportunity to compare the difference in numbers of pneumococci in washed and unwashed areas of cortex after a single large lavage, followed by optochin.

Dog 17.—Fox terrier, female; weight 5 kilos. March 30, 1927, 4.40 p.m., ether; cistern puncture; clear fluid. Injected 5,200,000 Type I pneumococci (6 hour dextrose broth culture; small transplant; growth 260 million per cc.). Good recovery.

24 hours—temperature 100.7°; in bad condition; irritable; unable to stand; convulsive. Morphine; ether; combined cistern and lumbar punctures; fluid cloudy; cells fairly numerous; hundreds of cocci per field. Lavaged from lumbar to cistern 50 cc. warm saline followed by 7 cc. 0.2 per cent optochin. On the 7th cc., respiration ceased, pulse continued strong, but short period of artificial respiration failed to revive; a drug death. Autopsy showed the cord, cistern, and cerebellum covered with a moderate film of exudate; a small triangular area of cortex just above the cerebellum was similar in appearance, but the base and balance of the cortex, where no lavage had penetrated, were overlaid with thick yellow pus and myriads of cocci. There was moderate bacteremia. Figs. 6 and 7 are offered for comparison of smears from washed and unwashed cortical areas. We are, of course, assuming that the initial distribution of cocci was the same.

This animal died from the effect of the drug on the respiratory center, and the manner of death typifies that which we have regularly seen with overdoses of drug. Respiration suddenly becomes shallow and slow, but pulse continues strong until some time after breathing has ceased. This effect on the respiratory center is transitory, and if artificial respiration is vigorously applied, with, if necessary, the addition of intracardiac adrenalin, it is almost always possible to revive the dog. When large concentrations of drug were used, respiratory difficulties were common, but deaths were few; artificial respiration was in two instances maintained for 20 minutes, during which time the

pulse ceased, only to be renewed by adrenalin injections. Recovery ensued.

A word may be said about the drug concentration which has seemed safe. This has been found not to exceed that present in a mixture of 15 cc. of antiserum and 0.75 cc. of 1 per cent optochin. When no antiserum is used, this dose is too high; a safe maximum has not been determined for drug not diluted with serum. If much pus is present, an animal may withstand higher concentrations of drug than would otherwise be the case; if lavage is very effective and the return fluid practically clear, the drug concentration should never exceed that given above. Great caution should be observed if the fluid return through the cistern needle is blocked. With excellent drainage, the drug effect on the respiratory center appears a function of percentage concentration of optochin in the lavaging fluid, rather than one of total cc. of mixture lavaged through the meninges.

Having determined the dosages of optochin-serum mixture which were tolerated with little or no respiratory disturbance, systematic treatments were again undertaken. The results are evident from the following representative protocols. For convenience, we are illustrating the type of result obtained in mild initial infections and comparing these with that secured in severe initial infections.

Dog 25.—Female hound; weight 7¼ kilos. April 25, 1927, 3.45 p.m., ether; cistern puncture; clear fluid. Injected 10,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 800 million per cc.). Good recovery.

19 hours—temperature 103.4°. Sluggish; has eaten. Morphine; ether; lumbar and cistern punctures; fluid cloudy; contains 15–20 cells per o. i. f., and about 1 diplococcus to every 3 fields; lavage attempted, but fluid came through with great difficulty and was blood-tinged. Lavage consequently abandoned. After drainage a mixture of serum 10 cc. and 1 per cent optochin 0.5 cc. was distributed equally between cord and cistern. Double frontal trephines were done and 3 cc. of a similar mixture introduced on each side. Cistern culture, abundant growth.

42 hours—temperature 101.3°. Sluggish; lateral nystagmus (traumatic?). Morphine; ether; double puncture; yellow opalescent fluid; cells 15 per o. i. f.; only 1 coccus seen; culture grew but 2 colonies. Injected 10 cc. saline with poor recovery; followed by 5 cc. optochin-serum mixture in lumbar subarachnoid space, and 2 cc. in each frontal. Good recovery.

66 hours—temperature 99°. No treatment. Subsequent uneventful recovery.

Dog 26.—Female hound; weight 5¼ kilos; a companion dog to the preceding. Infected with 8,000,000 pneumococci from the same culture.

20 hours—temperature 102.9°. Sluggish. Morphine; ether; quadruple puncture. Cisternal fluid cloudy; cells 15–30 per o. i. f.; cocci average 1 to every 2 fields. Saline lavage lumbar to cistern successful at first, but return then failed; repeated washing and draining *via* cisternal needle. Injection of mixture 15 cc. serum and 1.5 cc. 1 per cent optochin, divided 5 cc. cord, 5 cc. cistern, 2 cc. each frontal. Culture, abundant growth.

42 hours—temperature 102°. Clinically normal; usual double puncture, lumbar and cistern. Fluid clear; contains 2–3 cells per field, about half small lymphocytes. Introduced 6 cc. serum into lumbar subarachnoid space and placed head downward. No drug; no frontal treatment. Culture sterile.

66 hours—temperature 101°. No treatment.

Remained unchanged until the 16th day. Found prostrate and convulsive; snapped and salivated and was thought to have rabies. Chloroformed. Typical pneumococcus meningitis, generalized over convexity and base; cord relatively free. Smears show large numbers of pus cells and diplococci; fairly good phagocytosis. Ventricles dilated; purulent ventriculitis. Type I pneumococci recovered from all regions including even the ethmoid cells.

Summary: Mild initial process; sterilization incomplete; reinfection delayed and from some focus so small that in the period between the first treatment and the cistern tap, 24 hours later, no reinfection of the cisternal fluid had occurred, thereby giving a false negative culture.

The subsequent protocol is illustrative of the importance of residual foci in reinfecting meninges not completely sterilized. Experiments such as this are the type which convince us of the inefficacy of partial lavages as practiced by Kolmer.

Dog 20.—Female airedale; weight 9¼ kilos. Has canine distemper. April 6, 1927, morphine; ether; cisternal puncture; clear fluid; injected 10,500,000 Type I pneumococci (6 hour dextrose broth culture; growth 1,200,000,000 per cc.). Good recovery.

19 hours—temperature 102.8°; lethargic; morphine; ether; lumbar and cistern punctures; fluids purulent; contain several hundreds of cells and hundreds of pneumococci per o. i. f. (Fig. 8). Essentially no phagocytosis; a very severe infection. Lavage lumbar to cistern with 30 cc. saline followed by 11 cc. of mixture of serum 10 cc. and 1 per cent optochin 0.5 cc. Double frontal trephine with injection of 3 cc. of similar mixture on each side; 10 cc. of 800 unit antibody solution (Felton) intravenously; spinal fluid culture, confluent growth.

24 hours—temperature 100°. Ether; double puncture; lumbar and cistern; fluid clearer; cells 50 per o. i. f.; some contain phagocyted pneumococci (Fig. 9); practically no free organisms; culture, scattered colonies. Lavage lumber to cistern with 10 cc. saline followed by 0.035 per cent optochin-serum 7 cc.; no frontal treatment.

43 hours—temperature 98.8°; sluggish but otherwise unchanged; morphine; ether; lumbar and cistern punctures; fluid yellowish, opalescent; cells 10–35 per o. i. f.; no organisms seen. Injected 6 cc. serum, lumbar subarachnoid space and placed head down. 75 cc. intraperitoneal saline. Culture yielded about 300 colonies from a 0.5 cc. planting.

65 hours—temperature 102.7°; quite ill; nose a mass of thick pus; cistern and lumbar punctures; fluid a culture of pneumococci, hundreds per field (Fig. 10); cells rare; no agglutination nor phagocytosis. Represents a massive reinfection of a nearly sterile region, following cessation of complete treatments. Injected 8 cc. 0.05 per cent optochin-serum mixture divided between cord and cistern and 3 cc. into each frontal.

90 hours—temperature 99.6°; weaker; able to stand; ataxic; no change in distemper; ether; cistern and lumbar punctures; no fluid obtained. Gave 5 cc. lumbar, 1 cc. cistern, 3 cc. each frontal of 0.1 per cent optochin-serum. Intraperitoneally 150 cc. 10 per cent glucose; milk by stomach tube.

114 hours—temperature below 94°; chloroformed. Autopsy: Material suggesting chicken fat clot in fourth ventricle, extending down over cervical cord. Explains the last "dry tap." Fibrin at base, about pituitary and optic chiasm. Cord elsewhere relatively clear; anterior convexity relatively clear; meninges over posterior convexity opaque; exudate purulent; some pus in lateral ventricles.

Region	Cells per o. i. f.	Cocci	Culture
Cord (lumbar)	5–10	Rare phagocyted; very rare free	0
Cistern	20–75	Moderate; free and phagocyted	+
Anterior convexity	5–25	Only 2 cocci in entire smear	0
Posterior " (Fig. 11)	75	Rare; phagocyted	+
Base	25	Moderate; free and phagocyted	+
Lateral ventricle (Fig. 12)	75	Moderate, phagocyted; more, free	+

A pneumonia practically lobar in distribution, entire left lung; culture, *bronchisepticus*. Negative heart's blood.

Summary: Initial massive infection; tendency to sterilization with treatment; "reinfection" with cessation of treatment; renewed tendency toward sterilization with resumption of treatment. Main focus of infection lateral ventricle; course of disease interrupted by fatal outcome of distemper.

As examples of severe infections sterilized slowly and progressively by successive complete treatments in one instance, seven in all, necessitating altogether ten etherizations, partially for treatment and partially for diagnosis, the following may be offered.

Dog 29.—Male hound; weight 7¼ kilos. May 2, 1927, 4.00 p.m., morphine; ether; cistern puncture. Clear fluid. Injected 10,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 680 million per cc.). Good recovery.

24 hours—temperature 102.2°. Sluggish; lumbar puncture; fluid under increased tension, opalescent, yellowish; cells 50 per o. i. f.; diplococci 5–12 per field. Not treated. Culture, confluent growth.

42 hours—temperature 103.7°. Sluggish but able to stand. Morphine; ether; quadruple puncture; lumbar fluid yellowish, opalescent; cells 8–10 per o. i. f.; diplococci about 100 per field; similar cisternal fluid (Fig. 13). Lavage attempted, but proceeded with difficulty and with poor return through cisternal needle; abandoned. Injected 7 cc. of mixture of serum 10 cc., optochin 1 per cent, 0.5 cc. cisterna, 3 cc. lumbar, and 2 cc. similar mixture in each frontal subarachnoid region. Cultures, confluent growth.

66 hours—temperature 102.9°. Clinically unchanged. Morphine; ether; quadruple puncture. Cisternal fluid clearing; cells rare; diplococci less than 20 per field. Lavaged lumbar to cistern with 10 cc. of saline; injected serum-optochin mixture as above. Gave 100 cc. intraperitoneal glucose. Culture, confluent growth.

90 hours—temperature 102.8°. Unchanged. Quadruple puncture; fluid more cloudy; cells 20–40 per field; cocci double in number. Last treatment repeated. No lavage. Culture, confluent growth.

96 hours—treatment repeated with exception of frontal injections. Cisternal fluid cloudier; cells increased but what few pneumococci are present are all phagocyted. Culture, few colonies.

114 hours—temperature 102.6°. Clinically unchanged. Morphine; ether; cisternal puncture; fluid scanty; 6–7 diplococci per field; culture, colonies increased. Complete treatment as above with exception of left frontal; no lavage.

138 hours—temperature 104°; unchanged; has eaten and drunk; stands and wags tail. Ether; quadruple puncture; lavage lumbar to cistern with 15 cc. serum-optochin mixture (serum 15 cc., optochin 0.75 cc. of 1 per cent solution); 1.5 cc. same mixture, both frontal trephines. Fluid clearing; cells 5–10 per field; no cocci seen. Culture, scanty growth.

162 hours—temperature 103.7°; canine distemper; less active; will not stand without assistance. Ether; lumbar and cistern punctures; cells 4-5 per field; no cocci; *culture, sterile;* lavage with 14 cc. serum-optochin mixture as above. Intraperitoneal glucose, 100 cc.

186 hours—temperature 102.7°; distemper worse; thick pus flowing from nostrils. No treatment.

210 hours—unchanged; diagnostic cistern puncture; rare cells; no cocci; injected 5 cc. of serum as prophylactic; culture, negative.

288 hours—very ill from distemper. Subnormal temperature. Chloroformed. Bilateral bronchopneumonia (*B. bronchisepticus*). Entire brain and cord grossly normal save for slight excess of small lymphocytes and endothelial leucocytes in

cisternal and ventricular fluids. Cultures taken from all regions, including the entire fluid contents of the lateral ventricles, failed to give growth.

Microscopically some slight generalized infiltration of the meninges by endothelial leucocytes, many fatty, a few with blood pigment; a typical late clearing up stage. In addition, collections of lymphocytes in the region just beneath the ependyma of ventricles. An organizing (purpuric?) hemorrhage of gray matter of cord.

Dog 32.—Female hound; weight 5 kilos. May 4, 1927, 4.05 p.m., morphine; ether; cistern puncture; clear fluid. Injected 7,500,000 Type I pneumococci (6 hour dextrose broth culture; growth 750 million per cc.). Good recovery.

22 hours—temperature 104°; sluggish; irritable. Ether; lumbar puncture; fluid seropurulent; cells 15–20 per o. i. f.; cocci upwards of 150 per field (Fig. 14); cisternal fluid opalescent; cells rare; cocci 20–30 per field. Lavage, lumbar to cistern with 15 cc. saline. Frontal trephines; injection of mixture of 15 cc. antiserum and 0.75 cc. 1 per cent optochin as follows: lumbar subarachnoid space, 7 cc., cisterna magna, 3 cc., each frontal trephine, 2 cc. Culture, confluent growth.

42 hours—temperature 102.4°; stands weakly; attempts to walk; drinks. Ether; quadruple puncture; cisternal fluid clearing; cells 20–30 per field; no cocci seen; culture, colonies numerous but growth not confluent; injected serum-optochin mixture (as above) 7 cc. lumbar, 3 cc. cistern, 1 cc. each frontal.

66 hours—temperature 101°; marked loss of weight; weakness hind legs. Ether; quadruple puncture; fluid almost clear; cells 2–3 per field and cocci about 1 in every 2 or 3 fields; culture, colonies increased. Treatment as at 42 hours. Intraperitoneal glucose.

90 hours—temperature 98°; definite partial paraplegia hind legs; probably traumatic. No treatment.

114 hours—temperature 98.6°; unchanged; diagnostic cistern puncture; abundant crystal clear fluid; lymphocytes less than 1 per field; no organisms. Plated 2 cc. of fluid; no growth.

138 hours—temperature 99.3°; diagnostic puncture; negative culture. Subsequent course uneventful; persistent partial paraplegia.

The following may be offered as an example of a case where two complete treatments failed to sterilize the meninges appreciably. The condition was complicated by distemper.

Dog 34.—Young female collie pup. May 10, 1927, 4.00 p.m., ether; cisternal puncture; clear fluid. Injected 9,000,000 Type I pneumococci (6 hour dextrose broth culture; growth 610 million per cc.). Good recovery.

19 hours—temperature 104°; sluggish; has developed severe distemper.

23 hours—ether; quadruple puncture; cistern and lumbar fluids seropurulent; cells 50–75 per field; diplococci very numerous. Lavage lumbar to cistern with

20 cc. saline, followed by lavage with serum-optochin mixture (serum 15 cc., 1 per cent optochin 0.75 cc.), lumbar to cistern 15 cc.; frontal trephines 2 cc. each of mixture of similar proportions. Intraperitoneal glucose, 100 cc. Culture, confluent growth.

42 hours—temperature 101.5°; unchanged. Quadruple puncture; complete treatment as above; 100 cc. glucose intraperitoneally. Smear: an 80 per cent reduction in organisms; cells average 12 per field. Culture, confluent growth.

66 hours—temperature below 94°. Chloroformed. In gross the brain and cord show only a trace of exudate; microscopically smears contain relatively few cells; diplococci present in large numbers and save in the cord region, where there is some phagocytosis, no evidence is seen of beneficial effect of treatment. Marked purulent bronchitis, but death is undoubtedly meningeal. Cultures all give confluent growth; heart's blood, rare colonies. Histologically severe, diffuse leptomeningitis.

Comments.

Since optochin is somewhat under suspicion in view of its apparent tendency to produce transient amblyopia, we have endeavored to detect visual disturbances in recovered dogs. So far as was ascertained, none occurred; no ophthalmoscopic examinations were made. Some of the recovered dogs are deaf, but we know that in meningococcal meningitis permanent deafness may result.

A word may be said about lateral ventricular punctures. This has not been done in the dog; it is fairly certain that sterilization would be hastened and that more recoveries would have resulted had this been a routine procedure. Whereas the lateral ventricle of a dog, in the region where puncture is desirable, is a mere slit unless pathologic dilatation has occurred, it should offer no great problem in man. It is apparently the most difficult region to reach with serum by the methods applied in this study. In this respect the base in the region of the chiasm shares with the lateral ventricle and in case these methods should ever be employed in treating human disease, failure to sterilize by the quadruple puncture route should make one think seriously of ventricular punctures and punctures through to the base. The important thing in securing a cure is contact of all regions with the optochin-serum mixture—contact complete and frequently repeated. Undoubtedly lavage is a valuable adjuvant, but lavage alone cannot rid the meninges of organisms and if even a very small number remains, our experience shows that severe reinfection is almost inevitable. The object of treatment should be to wash out as many

organisms as possible and to control the residual bacteria by a pneumococcidal drug and a phagocytosis-producing antiserum.

One of the most hopeful and surprising things observed in the entire study was the manner in which lavage may be accomplished even in the presence of a massive exudate, it being possible to lavage a cord in an animal having almost frankly purulent cisternal and lumbar fluids, until the return fluid is nearly crystal clear; full cognizance is, however, taken of the fact that no matter how long one washes, within reason, cells and pneumococci blocked in fibrin meshes, the former certainly in large numbers, will remain. It has seemed that it is easier to lavage out organisms than it is cells, since the latter are larger and the network of fibrin offers more resistance to their free passage. As judged by the pictures of phagocytosis in foci fairly distant from the site of injection, the immune serum penetrates the exudate very satisfactorily.

No statistics of cures are presented. In view of the prevalence of traumatic deaths after frequently repeated cistern punctures, drug deaths, when high optochin concentrations were used, and especially mortality from epidemic canine distemper, such statistics would be meaningless. The fact that progressive sterilization of the meninges by methods employed in this study is possible has been established in our opinion without question.

Pathology.

The microscopic pathology has been studied in all animals dying of the disease, and in certain recovered dogs which succumbed to intercurrent disease (distemper) after becoming sterile. Sections show a rapidly spreading, fibrinopurulent leptomeningitis, which as early as 23 hours after infection may involve all regions of the meninges (Fig. 15). In a control dog, or in a treated animal in which the disease was not checked, the exudate reaches large proportions; the cord dura is invaded; the spinal nerve roots are involved (Fig. 16); there is an inflammatory process in the epidural fat (Fig. 17). Invasion and destruction of the choroid plexuses, superficial encephalitis, spread *via* the Virchow-Robin spaces are common (Figs. 18–20). An empyema of the third or fourth ventricle with extension into the central canal of the cord may occur (Fig. 21). Probably, too, the latter may

be reached from the ventral fissure. Once in the central canal the process may destroy the ependyma and invade the region of the commissures, and from thence the cornua by following a perivascular route, giving rise to a myelitis. Invasion, at least to any great extent, of the lateral ventricles occurs relatively late, probably because of the direction of flow of the spinal fluid; in other words, the lateral ventricles lavage themselves. With the development of a blocking exudate over the convexity or in the fourth ventricle or aqueduct, empyemas with dilatation of the lateral ventricles inevitably result. With the subsiding of the process, polymorphonuclears disappear and are replaced by fatty and phagocytic endothelial leucocytes and small lymphocytes, which cells apparently may persist for some time. Such was the picture observed in Dog 29, Fig. 22 (see protocol). Slight subependymal and peripheral cortical gliosis may be found.

Reinfection Experiments.

Five recovered dogs, together with a control, were reinfected to see if any immunity to meningeal infection existed. These animals (Dogs 1–5) were reinoculated 103, 79, 63, 47, and 42 days respectively after primary infection. Four ran an atypical course. The first two developed a very high grade, early leucocytosis; in one of these no organisms were observed in the 24 hour spinal fluid smear, and a culture gave no growth. Dog 1 died of severe purulent meningitis in 114 hours. Dog 2, whose culture was sterile 24 hours after infection, showed organisms at 66 hours; the number of cocci in the smears increased very slowly and at 7 days they were no more numerous than frequently seen after the 1st day in a primarily infected animal. As organisms increased, leucocytes diminished; phagocytosis was rarely seen and can scarcely account for the slow development of the disease. In none of the dogs were agglutinins demonstrable in the spinal fluids. Dog 2 died the 9th day. Dogs 3 and 5 gave negative cultures, and smears contained but 20–30 leucocytes per field 24 hours after infection; by 66 hours fluids were crystal clear and sterile. Dog 4 ran a typical course, but was sterilized by two complete optochin-serum treatments. This sterilization was unusually rapid. The control dog died typically in 48 hours. Hence a degree of increased resistance remained within the meninges.

RÉSUMÉ AND CONCLUSIONS.

1. Extensive acute, fibrinopurulent meningitis may be produced in dogs by the intracisternal injection of virulent Type I pneumococci.

2. Given an equal number of virulent infecting organisms, the rate of establishment of infection depends upon the phase of growth and the quantitative growth per cc. of culture plus an uncontrollable individual factor in the animal.

3. The pathology of pneumococcal meningitis is discussed. It resembles very closely the similar disease in man.

4. Systematic lavage and treatment with optochin-serum mixtures by the method of quadruple puncture, as described above, have resulted in cures of Type I pneumococcal meningitis in dogs.

5. The important factor in obtaining cures is to bring all regions of the meninges into frequent contact with the therapeutic agent.

6. In the absence of such contact, incomplete sterilization results and "reinfection" is almost inevitable.

7. Protocols show the necessity of repeated negative cisternal fluids, both on smear and on culture, before sterilization can be assured.

8. Recovered dogs subjected to meningeal reinfection show some degree of resistance.

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EXPLANATION OF PLATES.

PLATE 16.

FIG. 1. Control Dog 3. Massive basal exudate.

FIG. 2. Control Dog 7. Smear from convexity. × 1000.

FIG. 3. Distribution of dye introduced *via* left frontal trephine.

PLATE 17.

FIG. 4. Treated Dog 10. Dilated ventricles; purulent ventriculitis.

FIG. 5. Treated Dog 10. Ventricular smear showing extensive phagocytosis after ethylhydrocupreine hydrochloride treatment. × 1000.

FIG. 6. Treated Dog 17. Smear from posterior (lavaged) cortex. × 1000.

PLATE 18.

FIG. 7. Treated Dog 17. Smear from anterior cortex unaffected by lavage and chemo-serotherapy. × 1000.

FIG. 8. Treated Dog 20. Initial 19 hour cisternal smear. × 1000.

FIG. 9. Same dog. 24 hour smear (after one treatment). × 1000.

FIG. 10. Same dog. 65 hour smear, showing result of cessation of treatment. × 1000.

PLATE 19.

FIG. 11. Same dog. Smear from posterior convexity taken at 114 hours. Rare phagocyted cocci. × 1000.

FIG. 12. Same dog. Lateral ventricle smear. × 1000. Lateral ventricle a residual focus of infection.

FIG. 13. Recovered Dog 29. Cisternal smear before treatment. × 1000.

FIG. 14. Recovered Dog 32. Cisternal smear before treatment. × 1000.

PLATE 20.

FIG. 15. Control dog, showing extent of exudate at 23 hours. × 50.

FIG. 16. Control dog. Massive exudate. Invasion of spinal nerve. × 50.

FIG. 17. Treated dog. Accidental death. Inflammatory exudate in epidural fat. × 50.

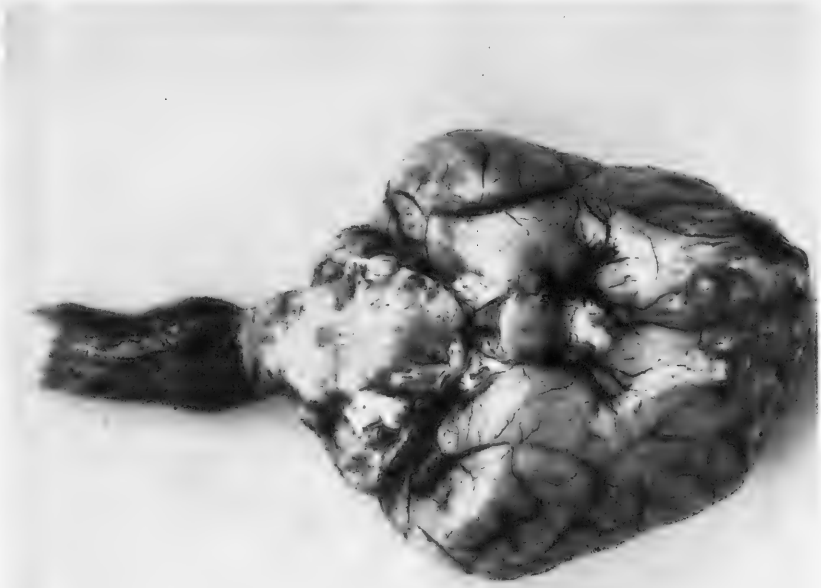
FIG. 18. Control dog. Purulent ventriculitis; superficial encephalitis; destruction of choroid plexus of lateral ventricle. × 50.

PLATE 21.

FIGS. 19 and 20. Control dog. Perivascular infiltration of cortex. × 50.

FIG. 21. Control dog. Empyema of central canal of cord; myelitis. × 50.

FIG. 22. Treated Dog 29. Sterile cultures; death from distemper; meninges essentially negative. × 50.



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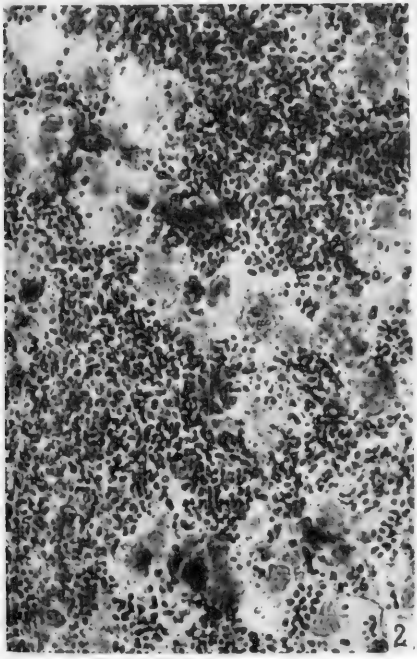

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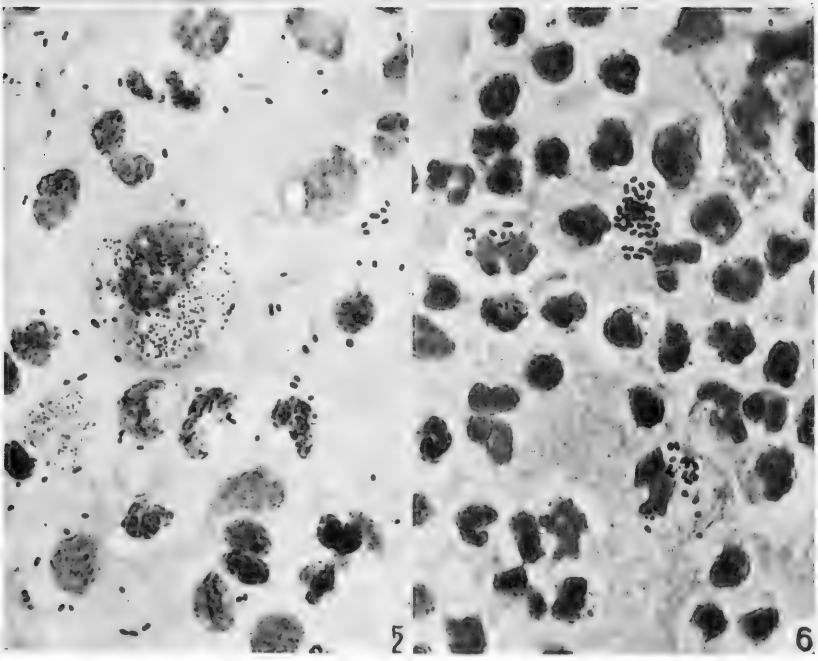

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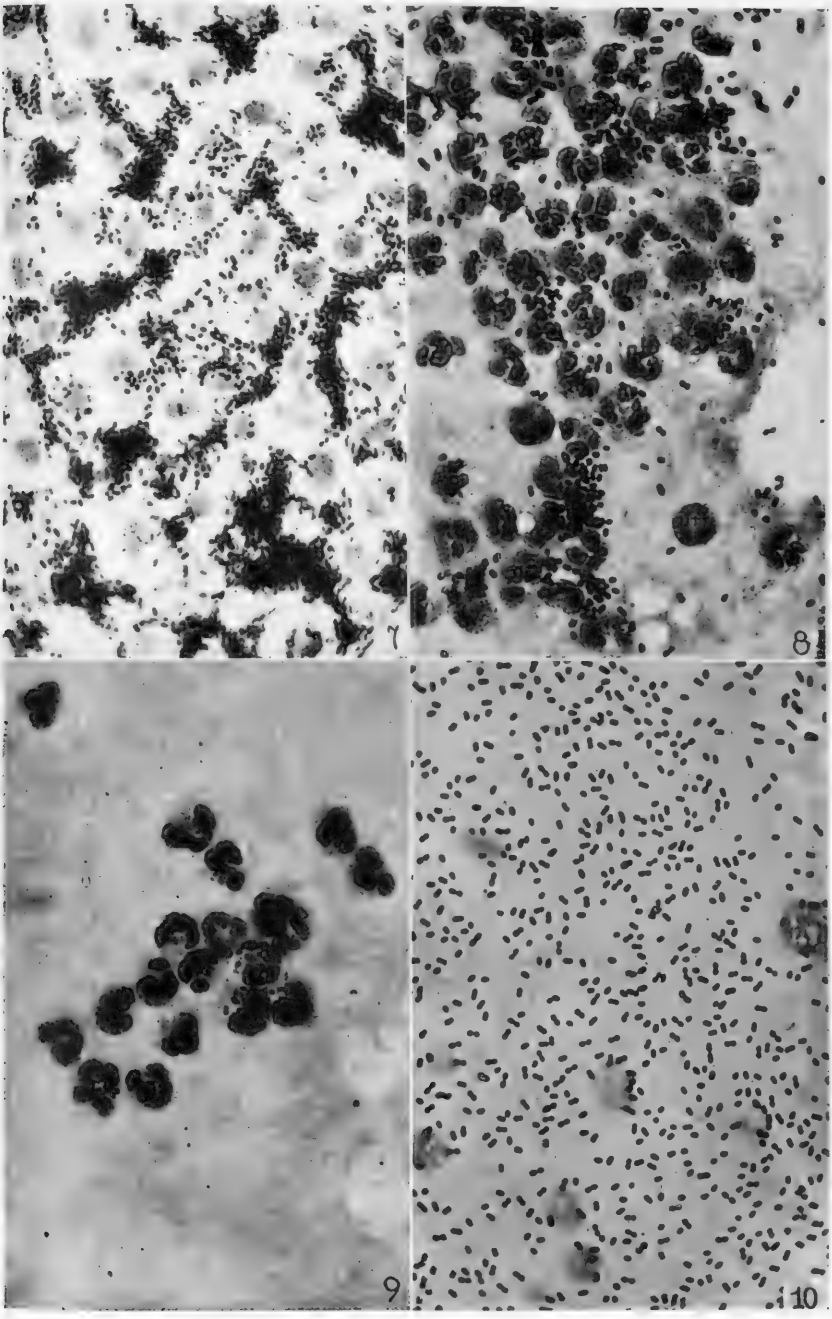

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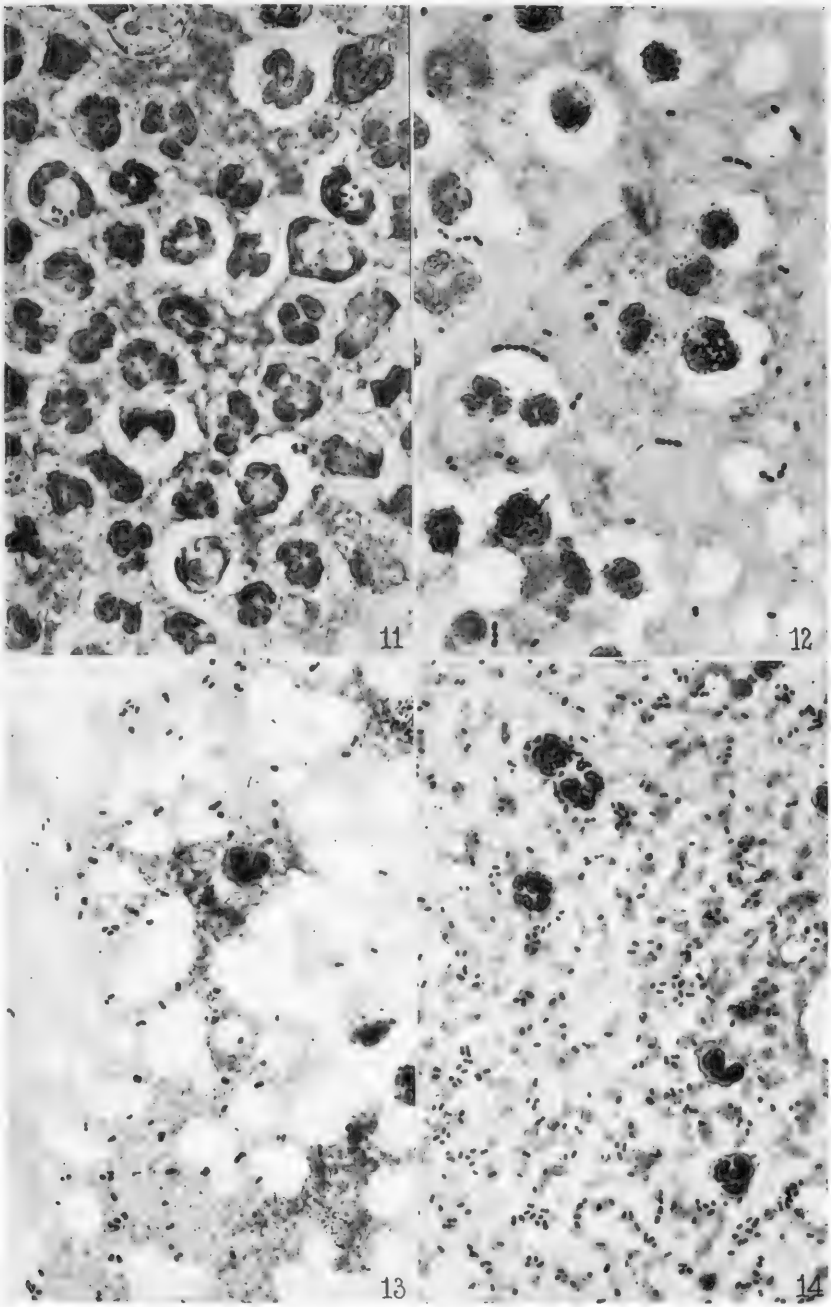

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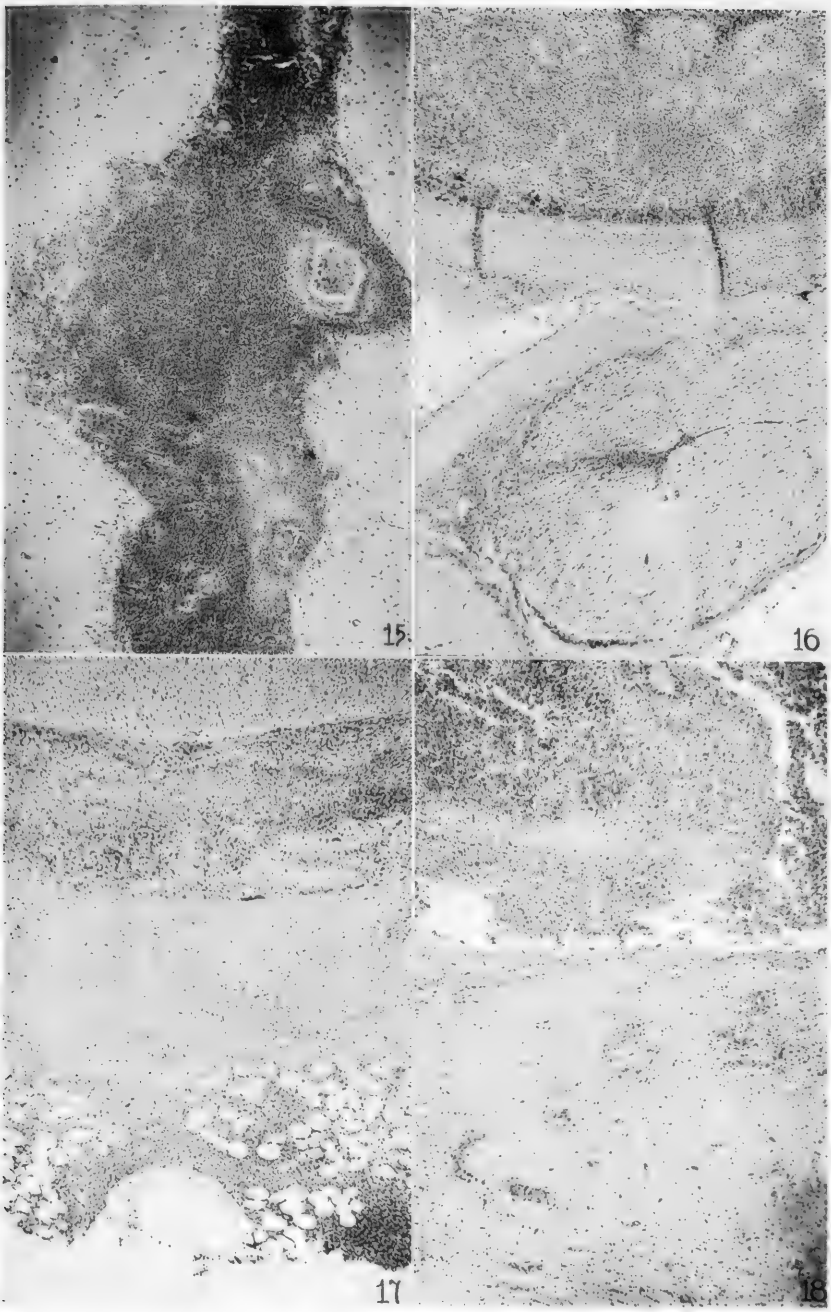

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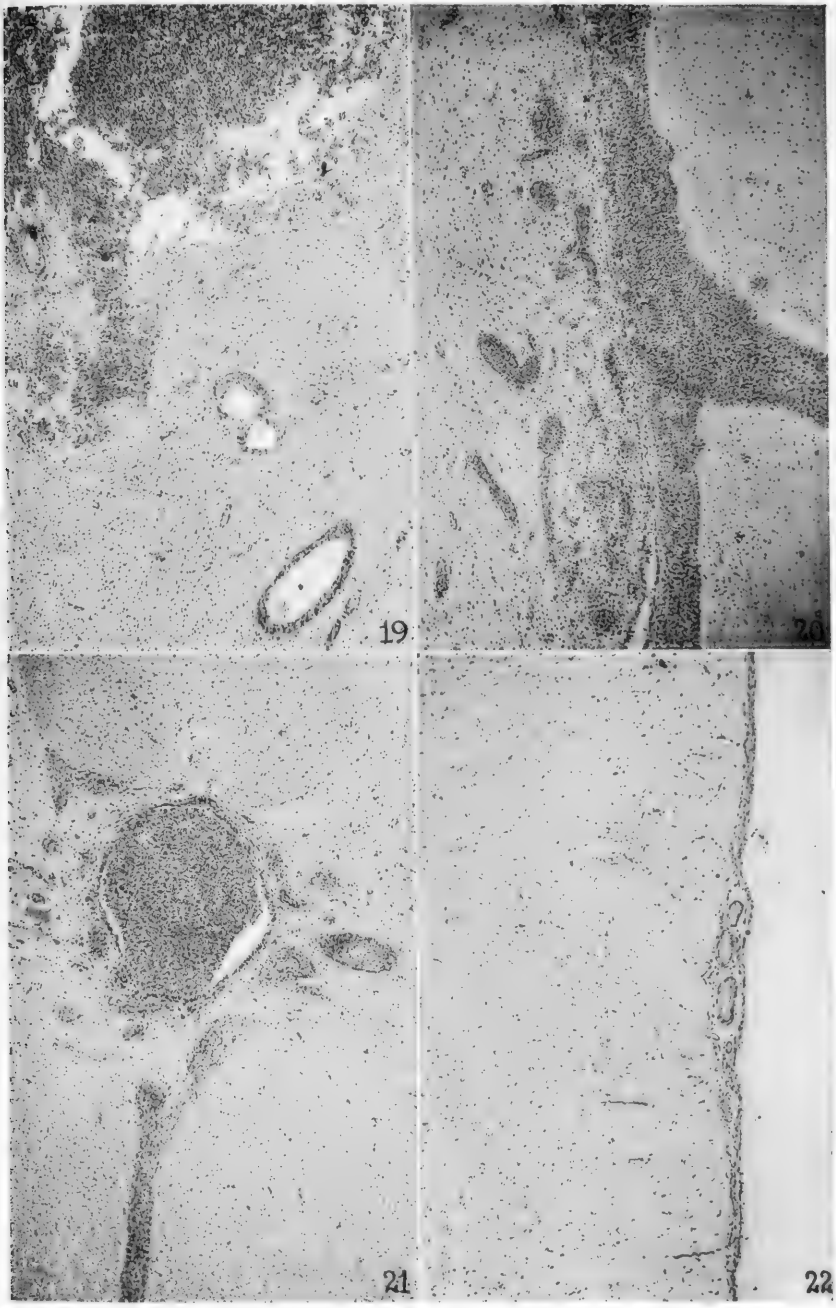

(Stewart: Pneumococcal meningitis. II.)

ENLARGEMENT OF THE ADRENAL CORTEX IN EXPERIMENTAL UREMIA.*

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Plates 22 and 23.

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The adrenal glands of a number of individuals who died in the uremia[1] of terminal inflammatory Bright's disease have been found to be larger than those obtained at postmortem following death from other causes. These patients usually if not always suffer from secondary infections and infections of various kinds have been found (1, 2) to cause enlargement of the adrenal glands. It is therefore difficult to say whether the adrenal enlargement is a result of the uremia *per se* or is brought about by secondary infections. The experiments which are described here were accordingly carried out to determine the effect in the rat of an uncomplicated experimental uremia on the weight and size of the adrenals.

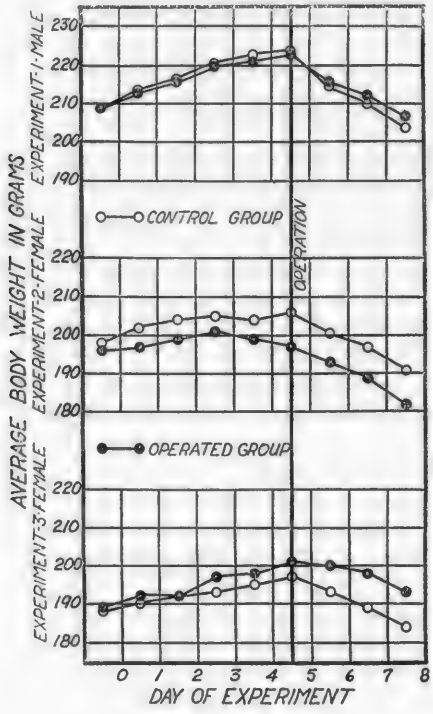
Methods.

Albino rats were used as the experimental animals. Because of the gross difference in the weights of the adrenal glands of the two sexes (3) a separate experiment was carried out on each sex. All the animals in an experiment were of the same sex and of exactly the same age. In each experiment the rats were arranged in order of their body weight, every alternate animal being placed in the control group. Both groups were put on a special diet and weighed daily for 5 days before operation. On the day of operation both kidneys were removed from each

* This work was aided by the Wellington Gregg Fund for the Investigation of Bright's Disease.

[1] The use of the term "uremia" is limited here to the cases having an abnormal retention of non-protein nitrogen in the blood and does not include patients suffering from so called uremic symptoms whose blood urea concentrations were not abnormal.

rat of the operated group through short lumbar incisions while the animal was under ether anesthesia. Within a very few minutes following the removal of both organs and the suspension of the anesthetic the animal was moving about its cage. The rats in the control group were treated in exactly the same manner as those undergoing the double nephrectomy except that their kidneys were only exposed. From the time of operation until they were killed the animals of both groups were without food but allowed water.



TEXT-FIG. 1.

The rats of the operated group were just as lively as those in the control group until about 70 hours after operation when the former would become comatose and show muscular twitchings. In order to avoid this terminal stage both groups were sacrificed about 60 hours after operation. Each rat was weighed and then anesthetized with ether. A blood specimen was obtained from the abdominal aorta and its urea content determined later. The adrenals were then removed,

carefully freed from adherent fat and connective tissue and weighed. This weight was recorded as the "wet" weight. The tissue was then fixed in 10 per cent neutral formalin.

The adrenals in each group were arranged in order of the magnitude of the adrenal weight, body weight, ratio. Every other pair of glands was imbedded in paraffin and sectioned serially. One 10 micron section from every 50 micra of tissue was preserved for determining cortex or medulla volume changes. The other half of the fixed adrenals of each group was washed 24 hours in distilled water and their "dry" weight determined after dehydration in a vacuum oven at 70°C. In one experiment the defatted dry weights were determined by extracting the dehydrated glands with hot anhydrous ether, an alcohol-ether mixture and finally absolute alcohol, several times in each case. The extracted glands were then further dried under reduced pressure and weighed.

Experiment 1 was performed with male rats 100 days of age at operation while in Experiment 2 female rats 180 days of age at the same time were used. The body weights for the two experiments are charted in Text-fig. 1. Neither the control nor operated groups lost very much weight during the 3 days that they were without food. Since in neither Experiment 1 nor 2 were the dried adrenals preserved for further treatment a third experiment was performed in order to obtain "defatted" adrenal weights. In this experiment female rats 170 days of age at the time of operation were used.

Results.

The results of Experiments 1 and 2 are given in Tables I and II. These give indisputable evidence of adrenal enlargement following the production of an experimental uremia by means of bilateral nephrectomy. Except for the inherent sex difference in the weight of the adrenals the results are essentially the same for both sexes. The actual differences between the averages of the fresh or "wet" adrenal weights of the two control and operated (uremic) groups are, for the males, 10.9 mg. and, for the females, 13.6 mg. while the probable difference of the average determined by the usual method is only $\pm$ 0.72 mg. for the males and $\pm$1.71 mg. for the females. The degree of adrenal enlargement in these experiments as determined by the "wet" weight of the glands 60 hours after the removal of both kidneys from the operated group amounted to 64.9 per cent in the male experiment and 46.8 per cent in the case of the female experiment.

Since the average initial body weights of the control and operated groups are practically alike the results (Tables I and II) determined from actual weights and adrenal weight in relation to the initial body weight are essentially the same. The latter only are considered here.

TABLE I.

Experiment 1 (Males).

No.	Body weight		Blood urea concentration	Adrenal weight	Adrenal tissue per 100 gm. body weight	
	Original	Final			"Wet"	"Dry"
	gm.	*gm.*	*mg. per 100 cc.*	*mg.*	*mg.*	*mg.*
			Control group			
1	190	179	30.8	36.1	18.9	—
3	216	210	26.3	40.0	18.5	6.4
5	190	180	30.9	35.0	18.4	—
7	204	194	27.3	37.0	18.1	6.8
9	200	196	32.4	35.3	17.5	—
11	208	202	30.0	35.8	17.3	5.8
13	210	216	27.2	35.0	16.7	—
15	226	221	31.7	36.6	16.4	5.6
17	214	206	28.1	29.0	13.5	—
19	230	231	33.6	30.2	13.0	5.0
Mean	209	204	29.8	35.0	16.8	5.9
			Operated (uremic) group			
2	204	204	550.0	68.0	33.3	—
4	212	219	574.6	68.1	32.1	10.5
6	198	198	556.8	56.9	28.8	—
8	220	216	551.0	61.7	28.2	11.2
10	180	173	700.0	50.0	27.8	—
12	196	193	550.7	51.6	26.5	10.1
14	206	208	566.0	54.2	26.2	—
16	224	220	487.5	56.0	25.0	8.1
18	238	240	570.0	59.1	24.7	—
20	212	204	549.0	52.0	24.5	8.6
Mean	209	207	565.6	57.8	27.7	9.7

It is conceivable that the enlargement of the adrenals which ensued in the uremic animals is a result not of hypertrophy but simply of an increase in weight brought about by an edema or at least an increase in water content. In each alternate animal in Experiments 1 and 2

(Tables I and II) and in every case in Experiment 3 (Table III) the glands were dehydrated by the methods which have been described and the "dry" weight of adrenal tissue determined. In Experiment 1 (males) the actual difference between the averages of the "dry"

TABLE II.

Experiment 2 (Females).

No.	Body weight		Blood urea concentration	Adrenal weight	Adrenal tissue per 100 gm. body weight	
	Original	Final			"Wet"	"Dry"
	gm.	*gm.*	*mg. per 100 cc.*	*mg.*	*mg.*	*mg.*
			Control group			
1	172	166	36.4	63.0	36.6	—
3	170	164	25.5	56.8	33.5	12.8
5	218	210	31.7	69.1	31.6	—
7	200	194	21.8	63.2	31.5	12.5
9	180	180	32.7	53.0	29.4	—
11	200	195	27.3	56.3	28.0	9.8
13	189	182	35.4	51.0	27.0	—
15	226	220	31.2	60.0	26.5	6.2
17	212	198	24.5	55.0	25.9	—
19	212	200	30.8	43.2	20.3	6.8
Mean	198	191	29.7	57.1	29.0	9.6
			Operated (uremic) group			
2	164	154	743.2	95.0	58.0	—
4	200	186	650.0	98.1	49.0	13.6
6	175	165	760.0	78.2	44.5	—
8	192	182	712.5	83.4	43.3	13.6
10	190	180	658.2	80.8	42.6	—
12	216	200	550.0	90.3	41.7	12.8
14	226	212	698.1	91.1	40.3	—
16	212	196	572.0	81.4	38.2	12.0
18	175	166	745.5	64.0	36.6	—
20	206	180	702.0	66.0	32.0	10.9
Mean	196	182	679.2	82.8	42.6	12.6

adrenal weights of the control and operated groups is 3.8 mg. while the probable difference is ± 0.40 mg. For Experiment 2 (females) the same figures are 3.0 mg. and ± 0.89 mg. and in Experiment 3 (females) 3.3 mg. and ± 0.59 mg. respectively. The degree of ad-

renal enlargement determined from the dry tissue weights in the three instances is 64.6 per cent (Experiment 1), 31.3 per cent (Experiment 2) and 32.7 per cent (Experiment 3). A large increase in weight is still present so that it is safe to conclude that an increased water content is not the sole cause of the heavy adrenals in the uremic rats. However since in neither case is the degree of enlargement as great as that found for the wet tissue weights the increase in water content was of somewhat greater magnitude than the degree of increase in solids. The increase in solids in the three experiments was 64.6 per cent, 31.3 per cent and 32.7 per cent respectively while the increase in water amounted to 65.1 per cent, 54.6 per cent and 77.3 per cent respectively. In one case (Experiment 1, males) the increases in solids and water content were the same.

Landau and McNee (4) have determined chemically the content of cholesterol and cholesterol esters in the adrenals for a number of conditions. Among these were several uremic individuals whose adrenal glands contained a higher percentage of cholesterol and cholesterol esters than any of the other cases examined. Because of this, and the interest which has often been manifested in the lipid content of the adrenals in relation to changes in these glands, in one experiment (Experiment 3) the glands were defatted with appropriate solvents to determine the possible increase in lipid content as a cause of the increased weight. The results in Table III indicate that substances soluble in fat solvents contributed more to the weight of the uremic than of the control adrenals, for the degree of hypertrophy determined from the dried defatted glands was less: 21.0 per cent as compared with the 32.7 and 31.3 per cent found for the dried glands which were not defatted.

As we have shown elsewhere (5) the compensatory hypertrophy of the remaining gland which follows unilateral adrenalectomy is limited to the cortex. The adrenal enlargement which follows chronic intoxication as in chronic infections (1) and after repeated injections of foreign proteins (2) in the production of antisera also appears to be confined to the cortex. In the present experiment likewise the hypertrophy was limited to the cortex. Alternate glands from Experiments 1 and 2 were available for histological examination. After fixing these were imbedded in paraffin and serial sections made.

TABLE III.

Experiment 3 (Females).

No.	Body weight		Adrenal weight	Adrenal tissue per 100 gm. body weight		
	Original	Final		"Wet"	"Dry"	"Defatted"
	gm.	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*
Control group						
1	170	172	68.3	40.2	14.7	10.8
3	174	174	59.8	34.4	12.9	11.8
5	164	162	49.8	30.3	7.6	5.5
7	194	176	57.7	29.7	9.3	9.2
9	168	168	47.0	28.0	9.1	6.0
11	204	184	57.0	27.9	10.8	10.1
13	180	182	49.2	27.3	9.2	7.8
15	180	182	48.6	27.0	9.7	7.8
17	186	176	50.0	26.9	9.7	9.6
19	206	196	54.6	26.5	9.9	4.8
21	214	210	56.0	26.1	10.2	9.0
23	198	190	48.9	25.2	10.6	8.2
25	200	202	49.9	25.0	9.0	7.7
27	189	190	47.1	24.9	9.3	6.3
29	200	192	48.1	24.0	9.4	7.2
Mean	188	184	52.8	28.2	10.1	8.1
Operated (uremic) group						
2	200	216	112.2	56.1	20.7	11.8
4	169	163	92.3	54.6	17.2	11.8
6	186	180	92.1	49.6	11.2	8.5
8	164	168	81.4	49.6	17.2	10.9
10	178	200	87.3	49.2	12.7	9.0
12	189	194	89.4	47.3	11.5	10.1
14	170	170	80.1	47.1	15.0	10.5
16	199	194	89.9	45.0	13.8	9.7
18	200	203	81.2	40.6	13.3	10.1
20	186	186	75.6	40.6	11.7	9.9
22	186	208	73.0	39.2	11.7	10.0
24	222	222	85.3	38.5	13.0	10.5
26	172	168	65.4	38.0	11.6	8.5
28	200	204	75.2	37.6	12.9	9.2
30	212	224	59.8	28.2	8.1	5.6
Mean	189	193	82.7	44.1	13.4	9.8

Precautions were taken during the imbedding to reduce the shrinkage to a minimum. Whatever reduction in size resulted was however probably of about the same degree in each group. Every fifth 10 micron section was accurately outlined by means of a camera lucida at a magnification of 35 diameters on heavy paper of uniform weight

TABLE IV.

Experiment 1 (Males).

No.	Adrenal volume	Medulla volume	Adrenal volume per 100 gm. original body weight		
			Whole gland	Medulla	Cortex
	c.mm.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
Control group					
1	7.8	0.7	4.10	0.37	3.73
5	5.7	0.5	3.00	0.26	2.74
9	7.7	0.8	3.85	0.40	3.45
13	8.0	0.7	3.81	0.33	3.48
17	5.4	0.4	2.52	0.19	2.33
Mean	**6.9**	**0.6**	**3.45**	**0.31**	**3.15**
Operated (uremic) group					
2	8.4	0.5	4.12	0.24	3.88
6	7.3	0.6	3.69	0.30	3.39
10	11.2	0.9	6.22	0.50	5.72
14	9.0	0.6	4.37	0.29	4.08
18	12.7	0.9	5.32	0.38	4.94
Mean	**9.7**	**0.7**	**4.74**	**0.37**	**4.40**

	Difference of means		Hypertrophy
	Actual	Probable	
			per cent
Whole gland.....	1.29	±0.33	37.4
Cortex.....	1.25	±0.30	39.7
Medulla.....	0.06	±0.11	(19.3)

per unit area. The medulla if present in the section was also outlined. Typical central sections through the glands of Experiments 1 and 2 are reproduced in Text-fig. 2. The area of these enlarged paper sections was determined by their weight. These areas of the whole gland and of the medulla, based on 10 micron sections, were

TABLE V.

Experiment 2 (Females).

No.	Adrenal volume	Medulla volume	Adrenal volume per 100 gm. original body weight		
			Whole gland	Medulla	Cortex
	c.mm.	*c.mm.*	*c.mm.*	*c.mm.*	*c.mm.*
Control group					
1	10.3	1.2	6.00	0.70	5.30
5	10.3	0.9	4.73	0.41	4.32
9	9.6	0.7	5.31	0.39	4.92
13	7.3	0.8	3.86	0.42	3.44
17	7.6	0.6	3.58	0.28	3.30
Mean	**9.0**	**0.8**	**4.69**	**0.44**	**4.25**
Operated (uremic) group					
2	14.4	1.2	8.78	0.73	8.05
6	13.4	0.6	7.66	0.34	7.32
10	13.1	0.8	6.90	0.42	6.48
14	14.1	0.9	6.22	0.40	5.82
18	12.6	1.1	7.20	0.63	6.57
Mean	**13.5**	**0.9**	**7.35**	**0.50**	**6.85**

	Difference of means		Hypertrophy
	Actual	Probable	
			per cent
Whole gland	2.66	±0.36	56.6
Cortex	2.60	±0.33	61.2
Medulla	0.06	±0.20	(13.7)

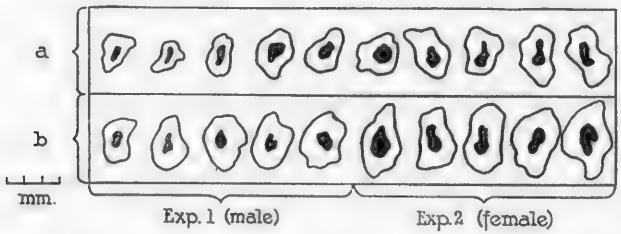
TABLE VI.

Percentage Increase of Adrenals in Uremia.

	Whole gland					Cortex	Medulla
	Weight	Solids	Water	Lipids	Volume	Volume	Volume
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Males	64.9	64.6	65.1		37.4	39.7	19.3
Females	46.8	32.0	65.9	80.0	56.6	61.2	13.7

considered representative of the 50 micron slabs from which they came and the volume of the 50 micron section thus determined. The volume of the cortex and medulla of each gland was then determined. These results comprise Tables IV and V.

It is evident from both experiments that the hypertrophy is limited to the cortex as there is no significant difference in the medulla volumes of the control and hypertrophied glands. In the case of the males when considered as hypertrophy of the entire gland, the increase in the weight of the tissue as we have already shown amounted to 65 per cent. This is due to a 40 per cent increase in the volume of the



TEXT-FIG. 2. Camera lucida outlines of central sections of adrenal medulla and cortex. (*a*) Normal adrenals of control groups. (*b*) Hypertrophied adrenals of uremic groups.

cortex. The increase in the weight of the whole gland in the female group (Table II) was 47 per cent and the increase in cortex volume 61 per cent. In Table VI these increases have been compared with the increases in weight and the chemical findings.

Histology.

The adrenal glands from the uremic rats have been shown to contain a higher percentage of material soluble in fat solvents than those from the controls. This is in disagreement with the observations of Pfeiffer (6) who found that the amount of fat demonstrable microscopically in the adrenals of guinea pigs and rabbits which were made uremic by double nephrectomy was much less than normally present. A number of adrenals from uremic and control rats were therefore examined in frozen section with a fat stain (Sudan III).

The pictures obtained (Fig. 1) were similar to those described by Pfeiffer and did not bear out our chemical findings. In both sets of glands the medulla as usual contained no fat while the zona glomerulosa of the cortex contained the most. Between these two zones there was a gradual variation in the amount present but throughout the fat stain was evident in a much higher concentration and more evenly distributed in any given zone in the normal adrenals. We must conclude consequently that although material soluble in fat solvents is present in higher concentration in uremic adrenal glands most of this is of such a nature that it does not take the ordinary fat stains. The differences in staining and solubility suggest (15) that the decrease in the uremic glands is in cholesterol while there is an increase in saturated fatty acids which do not take the fat stain Sudan III.

On microscopical examination of paraffin adrenal sections (stained with iron-hematoxylin and eosin) from uremic animals a number of changes from the normal picture were found present. As we have noted, the cortex is much larger (Text-fig. 2) in the uremic adrenals. In a number of cases there were areas of hyperemia in the cortex and in two glands small hemorrhages surrounded by an area of necrosis were found. Hyperemia, hemorrhage and necrosis of the adrenal cortex have been reported before (6, 8) as a sequence of experimental uremia in guinea pigs, rabbits and dogs. In our experiments it formed an almost negligible part of the histological picture.

The cortical cells (Fig. 2) of the uremic adrenals were greatly enlarged. Their columnar arrangement was obliterated. The swollen cells did not stain as deeply or with a granular protoplasm such as is present in the cells of the normal gland. Nuclei of all the cortical cells were swollen and larger than normal. They stained more lightly than usual and in the fixed section were surrounded by unstained areolæ. In the reticular zone of the cortex (Fig. 3) the same differences between the cells in the control and uremic adrenals were found. In addition this part of the uremic glands was vacuolated by many dilated capillaries lined with endothelium but containing no cells or other stainable material. Although potentially present in the normal gland they are not evident except under very high power.

The medulla (Fig. 4) of the uremic adrenals showed distended

capillaries similar to those present in the adjoining cortical area. It is possible that the higher degree of adrenal enlargement found with the wet weights than with the dried glands may have been due to the fluid congestion of these areas. As has been noted by others (7), there was also evidence of degeneration in the medullary cells. The nuclei were swollen and stained less intensely than before and there was (6–9) a decrease in the chromaffin staining substance. The cells of the medulla were swollen. It would be supposed that this cellular enlargement in addition to the distended sinuses would give larger adrenal medulla volumes in the uremic than in the control rats and such was the case in the two experiments (Tables IV and V) in which this was determined. The increase in the medulla volume was however so small that it had no statistical significance.

DISCUSSION.

There is little reason to believe that the changes which have been found in adrenal glands following the production of an experimental uremia are unique for this condition. They are probably the manifestation of an intoxication and follow uremia because of the abnormal retention of metabolites. The cortical hypertrophy which ensued is perhaps analogous to the marked hypertrophy of the adrenal cortex known (1, 2) to accompany chronic infections and intoxications in animals. A somewhat similar cortical enlargement follows foreign protein injections (2) in the production of antisera and we have recently found an increase in the volume of the adrenal cortex of the rat as a result of chronic intoxication with morphine or bacterial vaccine.

In severe infections and intoxications pathological changes in the adrenal glands (6, 10, 11) occur regularly, varying in intensity from hyperemia, congestion and edema to hemorrhages and foci of necrosis. Changes of a similar nature in the uremic adrenals have been described. A decrease in the chromaphil staining of the adrenal medulla accompanied the uremia. This exhaustion is common to various intoxications and infections (6, 12).

The characteristic stainable lipids in the adrenal cortex show the same striking alteration in distribution (irregular) and amount (less) in uremia as in other intoxications (6, 12, 13, 14).

SUMMARY.

The production of an experimental uremia in the albino rat by removal of both kidneys is followed by hypertrophy of the adrenal glands. In the case of male rats 90 days of age this adrenal enlargement amounted to 65 per cent and in the case of female rats 180 days of age 47 per cent.

The increase in the size of the whole gland is due entirely to hypertrophy of the cortex. This increase in the volume of cortical tissue amounted to approximately 40 per cent for males and 61 per cent for females and was due in large part to an increase in the size of the cells.

The content of water and material soluble in fat solvents was higher in the uremic than the control glands. However after subtraction of such storage materials a true hypertrophy of the cortex still remained. It amounted to 21 per cent.

Histologically the stainable fat had a more irregular distribution and was present in lesser amount in the adrenals from the uremic animals. The capillaries of the medulla and reticular cortex were distended. The nuclei of both the cortical and medullary cells were swollen and stained faintly.

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EXPLANATION OF PLATES.

PLATE 22.

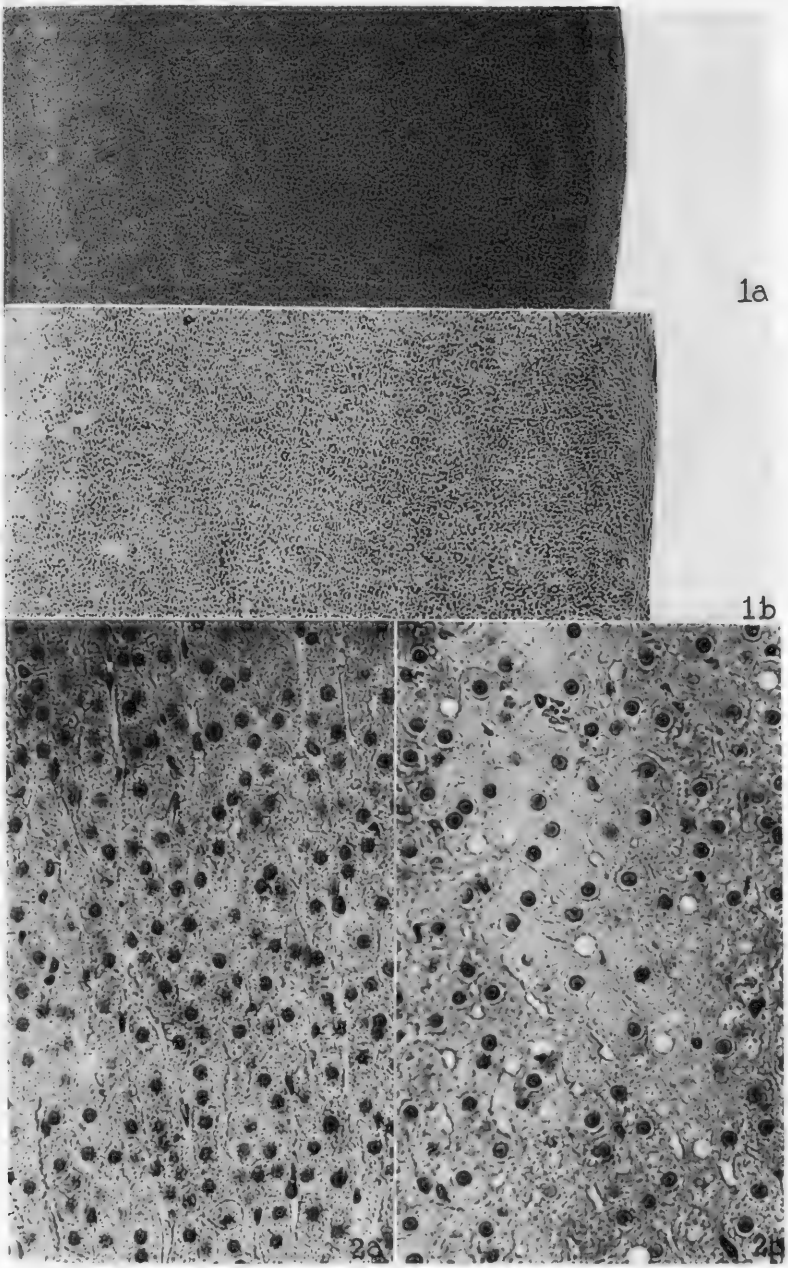
FIG. 1. Adrenal sections from (*a*) control rat, and (*b*) uremic rat. Cut, stained and photographed under identical conditions. 10 micron frozen sections stained with Sudan III. × 90. Shows the irregular distribution and diminished concentration of stainable lipids in the uremic adrenal cortex.

FIG. 2. Zona fasciculata of adrenal cortex, (*a*) control; (*b*) uremic. 6 micron paraffin sections stained with iron-hematoxylin and eosin. × 350. Shows the swollen nuclei and cells in the uremic gland.

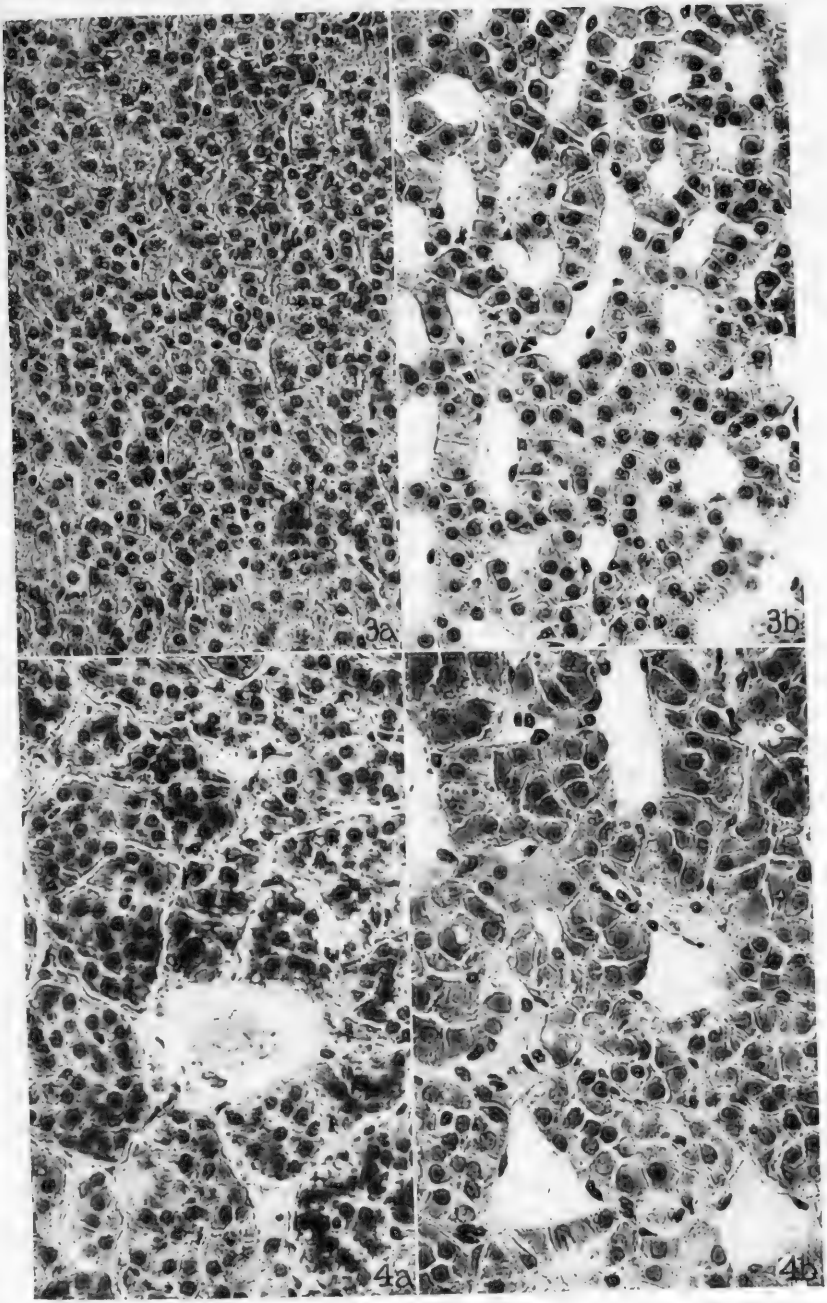
PLATE 23.

FIG. 3. Zona reticularis of adrenal cortex, (*a*) control; (*b*) uremic. 6 micron paraffin sections stained with hematoxylin and eosin. × 350. Shows distended capillaries.

FIG. 4. Adrenal medulla. (*a*) control; (*b*) uremic. 6 micron paraffin sections stained with hematoxylin and eosin after chrome fixation. × 350. Shows distended capillaries and large nuclei. The swollen cells contain a decreased amount of chromaffin substance.



(MacKay and MacKay: Enlargement of adrenal cortex in uremia.)



(MacKay and MacKay: Enlargement of adrenal cortex in uremia.)

THE SYSTOLIC BLOOD PRESSURE OF THE NORMAL RABBIT MEASURED BY A SLIGHTLY MODIFIED VAN LEERSUM METHOD.

Final Report.

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In this report it is intended to complete and extend my previous publication (1) on the systolic blood pressure of the rabbit. An account of the method used, together with the technique both of the loop operation and of the measurements, is to be found in the first report and will not be given here.

The number of loops made has been 186, the first one on April 4, 1923, the last one on July 30, 1926. Good loops, that is, even, soft and of convenient length, were obtained in 97 (52.2 per cent), of which 4 were lost before any measurement was made (trauma, pneumonia) and 3 are omitted now because the measurements were started in the course of an experiment. The majority of failures is due to necrosis or infection of the loop. From March 31 to April 16, 1925, 23 loops were made, of which only 3 were satisfactory (13 per cent), 18 being spoiled by necrosis or infection (78.3 per cent). On the other hand, from September 25 to September 30 of the same year, 22 loops were made, of which 16 were good (72.7 per cent), only 5 being ruined by infection or necrosis (22.8 per cent). On July 29 and 30, 1926, 7 loops were made, all good. These figures show the extremes of failure and success which have been met in this work. The success of the other series falls between 50 and 60 per cent, so that the poor result with the loops made in April, 1925, seems to have been due to some extraneous factor not determined. The other causes of failure are general, such as pneumonia, pleurisy, otitis media, etc., and are not peculiar to this plastic operation. There is still another complication which may

TABLE I.

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight		Remarks
										Initial	Final	
		1923		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
37-2	F	May 15–Sept. 12	92	126	165	102		272	172	2.110		Weight Apr. 4. Snuffles
39-6	F	May 15–June 1	11	125	136	90						Snuffles. Ear canker
39-7	F	May 16–June 5	15	130	170	125						Snuffles
42-0	F	July 25–Oct. 17	48	118	147	90	228	264	176		2.275	Albino. Litter of 10 Aug. 1
42-1	M	Aug. 3–Sept. 10	31	103.6	132	72	204	224	120	2.675		Weight June 5
46-1	M	Aug. 4–Oct. 17	40	104.1	130		208	256	160	2.390	2.600	Constriction. Snuffles
46-9	M	Oct. 16–Dec. 3	35	136.6	157	105	232	264	160	2.350	2.390	White Angora
		1923–24										
47-0	M	Aug. 4–Jan. 16	103	102.4	149	90	194	248	128	2.375	2.805	
		1923										
47-3	M	Aug. 18–Aug. 20	2	129.5	143	119	204	264	200	1.702		Weight July 24. Trauma
47-4	M	Oct.–Dec. 1	37	105.9	138	82	248	264	112	1.895	1.905	
47-7	M	Oct. 18–Nov. 26	31	101	133		162	236	136	2.120		Weight Oct. 27. Constriction. Abscess at angle of left lower jaw
48-2	M	Oct. 9–Dec. 1	44	111.9	129	75	242	264	156		2.060	
		1923–24										
48-3	M	Oct. 8–Oct. 13	266	109.5	144	77	222	280	112	2.290	3.145	Sciatic injury, Oct. 18, 1923
		1923										
48-4	M	Aug. 21–Oct. 12	16	114.1	127		240	300	208			Resistance. Abscess (flank). Constriction

48-5	M	Oct. 18–Dec. 1	35	139.5	156	92	253	296	160		2.120	Sciatic injury
48-7	M	Aug. 15–Dec. 1	58	128.1	172	103	296	300+	192	2.285	2.610	Albino. Mediastinal abscess during experiment
48-8	F	Aug. 14–Sept. 8 *1923–24*	23	110	143	94	156	240	148		2.585	Abscesses in abdominal wall
48-9	M	Aug. 14–Oct. 13 *1924*	301	134.5	201	116	248	300+	144	2.890	3.015	
D 1	M	Jan. 7–Feb. 26	44	99.2	135		200	220	136	2.310	2.855	Constriction
D 3	F	Jan. 7–Feb. 11	31	126.8	140	95	200	280	132	2.290	2.000	Purulent pleurisy
D 6	F	Jan. 7–Feb. 26	45	128.1	140		176	232	152	2.065	2.495	Constriction, marked
D 8	F	Jan. 7–Mar. 27	39	144.8	160	103	192	300+	176	2.795	2.610	
D 10	F	Jan. 7–Feb. 26	44	140.4	150		264	280	188	2.465	2.705	Constriction
D 12	F	Jan. 7–Feb. 26	44	129.3	132		224	224	152	1.970	2.435	Albino
D 13	M	Feb. 1–Mar. 27	46	138.7	157	95	192	240	136	2.345	2.275	
D 16	F	Apr. 15–Apr. 29	10	136	159	124	240	256	216	2.275	2.305	
D 19	M	Apr. 15–Apr. 29	10	143.6	162	125	232	256	184	2.060	2.050	
D 21	F	Apr. 15–Apr. 29	9	154.6	162	135	216	216	176	2.175	2.200	
D 22	F	Apr. 15–June 17	19	126.7	168	111	192	232	176	2.280	2.480	
D 23	F	Apr. 15–May 15	12	115.3	133		184	216	144	2.080	2.150	Constriction
D 24	M	May 1–May 15	10	121	129		208	232	168	1.845	1.915	Constriction
D 25	F	May 20–July 17	28	84.6	110	72	216	248	160	1.840	2.085	Resistance
D 26	F	May 1–May 28	21	128.2	148		200	220	168	1.975	2.225	Constriction
D 27	F	Apr. 22–May 20	20	122.9	135		216	240	184	1.815	2.000	Constriction
D 28	F	June 13–July 11	23	109.9	131	91	168	224	144	2.055	2.310	
D 31	F	Apr. 22–May 20	18	108	119		176	224	160	1.920	1.975	Constriction
D 33	M	Apr. 23–Apr. 28	5	109.6	120	87	200	224	168	1.885	1.865	Trauma
D 34	F	May 30–June 17	16	125.6	133	97	184	200	136	2.370	2.635	
D 41	M	June 12–July 10	23	137.1	145	96	192	232	164	2.325	2.290	
D 42	M	June 12–July 10	22	108	120	90	224	240	192	1.820	1.965	
D 45	M	June 5–July 11	29	107	122	89	208	240	152	2.125	2.060	

TABLE I—*Continued.*

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight		Remarks
										Initial	Final	
		1924		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
D 46	M	June 12–July 11	23	135.1	149	105	184	232	160	2.184	2.115	
D 49	F	June 30–July 19	16	116.7	140	97	184	232	168	2.140	2.365	
D 53	F	June 30–July 19	17	95.6	116	89	208	252	176	1.970	2.050	
D 55	F	June 30–July 19	17	123.1	155	107	216	248	168	2.425	2.215	Litter of 3 July 6
D 56	M	June 30–Aug. 22	43	101.7	132	84	208	248	160	1.955	2.165	Ear canker
D 59	F	June 30–July 19	17	115.8	145	98	196	232	176	1.910	1.965	
D 61	M	Aug. 11–Sept. 30	15	95.8	131	90	224	256	208	2.095	1.705	Sciatic injury
D 62	F	July 8–Sept. 10	28	129.6	154	102	216	256	168	2.415	2.315	Litter of 8 July 16
D 65	M	Aug. 12–Sept. 10	25	138.2	170	112	192	240	168	2.150	2.325	
D 66	M	Aug. 11–Oct. 27	61	100	125	70	208	280	144	1.945	2.460	Large abscess in thigh
D 67		Aug. 1–Sept. 10	25	97.6	129	92	248	264	176	2.420	2.635	
		1924–25										
D 69	F	Sept. 25–Feb. 10	76	145	174	126	224	248	160	2.825	3.460	
D 77		Nov. 10–Feb. 5	40	120.4	140	98	200	288	152	2.815	3.580	
D 78	M	Nov. 10–Feb. 5	39	114.5	156	91	264	280	168	3.115	3.730	Resistance. Constriction
D 80	M	Nov. 10–Mar. 24	69	144.4	168		216	240	152	2.965	3.040	Otitis media. Constriction
D 82	F	Nov. 10–Feb. 10	43	110	135	90	224	248	168	2.325	3.355	
		1924										
D 83	F	Nov. 10–Nov. 29	6	113.1	130	110	248	256	144	2.725	2.875	Chewed loop

		1924–25										
D 84	F	Nov. 24–Feb. 10	35	121.1	152	106	248	296	216	2.780	3.320	
D 85	M	Nov. 24–Feb. 10	33	103.7	141	99	200	248	144	2.820	3.350	
		1925										
D 87	M	Jan. 8–Mar. 24	59	110.8	130	82	264	264	148		3.625	Weight Oct. 24, 1924, 2.270 kg.
		1924										
D 88		Nov. 24–Nov. 26	3	109.6	115	94	200	200	176	2.480		Death from diarrhea
		1925										
D 89		Jan. 8–Mar. 26	60	118	142	97	224	248	160		3.195	Weight 2.300 kg. Nov. 22, 1924
		1924–25										
D 91	F	Nov. 24–Feb. 14	38	128.5	142		216	224	168	2.220	2.945	Constriction
		1924										
D 92	F	Nov. 24–Nov. 25	2	121.1	125	102	288	300	288	2.320		Accidental death
		1924–25										
D 94	F	Nov. 24–Feb. 10	34	126.3	167	112	232	288	200	2.310	2.460	
D 96	M	Nov. 24–Mar. 24	62	114.5	152		240	272	184	2.150	2.880	Constriction
		1925										
D 97	F	Apr. 15	1	133.5	140	130	248			3.480		
D 98	F	Apr. 15	1	131.1	138	124	272			2.550		
D 116	F	Sept. 24–Oct. 30	17	124.4	133	107	224	256	216		3.010	Constriction
D 118	F	Sept. 24–Oct. 30	16	123.9	138	95	232	256	208		2.330	
D 119	F	Sept. 24–Oct. 30	16	100.6	122	92	264	288	224		2.670	
D 123	F	Oct. 19	1	84.3	90	80	240			2.250		Weight Sept. 25, 1925. Trauma
D 125	M	Oct. 19–Oct. 30	10	101.7	119	89	232	240	216		2.320	Constriction (late appearance)
D 127	M	Oct. 19–Oct. 30	10	122.9	134	85	232	248	200		2.160	
D 131	M	Oct. 19–Oct. 30	10	97.4	112	72	232	240	184		2.080	
D 134	M	Oct. 20	1	71.3	75	69	208			1.720		Trauma. Weight Sept. 29, 1925
D 135	F	Oct. 19–Oct. 30	10	73.4	89	68	184	232	184		2.590	
D 136	M	Oct. 19–Oct. 30	10	82	105	80	176	208	176		2.510	
		1926										
D 137	F	June 10–June 22	9	114.2	129	102	192	208	176		2.920	

TABLE I—*Concluded.*

No. of rabbit	Sex	Observation period	No. of days on which blood pressure was taken	Mean of blood pressure 1st day	Maximum blood pressure whole period	Minimum blood pressure whole period	Pulse rate 1st day	Fastest pulse rate whole period	Slowest pulse rate whole period	Body weight		Remarks
										Initial	Final	
		1925–26		*mm. Hg*	*mm. Hg*	*mm. Hg*				*kg.*	*kg.*	
D 138	F	Oct. 19–Apr. 22	103	91.9	112	77	216	280	192	2.475	2.650	Constriction
		1926										
D 139	F	June 10–June 22	9	102.2	129	98	216	272	200		3.140	
		1925–26										
D 140	M	Oct. 19–June 22	111	101.7	132	74	200	264	184	2.065	3.410	
D 141	M	Oct. 19–Apr. 22	92	91	126	79	208	280	184	1.885	3.060	Sciatic injury, Nov. 21, 1925
		1926										
D 142	M	June 10–June 23	10	132.9	136	105	224	272	224		2.980	
		1925–26										
D 143	F	Oct. 19–June 22	107	106.3	138	85	240	288	216	2.310	3.470	
		1926										
D 144	F	Aug. 24–Oct. 4	10	100.7	115	90		240	208		2.565	
D 145	M	Aug. 24	1	90.4	92	89						Trauma
D 146	M	Aug. 24–Oct. 4	10	91.9	103	84	208	224	184		2.365	Accidental death
D 148	F	Aug. 24–Oct. 4	10	77.4	98	70	208	240	200		2.835	

appear when the animal kicks within the controlling box, and to which young animals, very quick in their actions and with soft bones, are specially susceptible. This is referred to below as "spinal trauma" and in Table I as "trauma." In some instances, under the same circumstances, instead of a complete paraplegia, there appears a partial

TABLE II.

Blood pressure	No. animals	Per cent
mm. Hg		
150–	1	1.1
140–149	6	6.7
130–139	13	14.4
120–129	20	22.2
110–119	18	20.0
100–109	16	17.8
90–99	10	11.1
80–89	3	3.3
70–79	3	3.3
	90	99.9

TABLE III.

Pulse rate per min.	No. animals	Per cent
280–299	2	2.3
260–279	5	5.9
240–259	13	15.3
220–239	17	20.0
200–219	29	34.1
180–199	13	15.3
160–179	5	5.9
140–159	1	1.2
	85	100.0

paralysis of one or both of the hind legs, without disturbance of the sphincters, and the animal recovers completely. This is referred to as "nerve injury." This complication is caused by the use of a controlling box and is not inherent in the loop method. One complication peculiar to the method is that some animals chew the loop. Five

did this: four (D 65, D 80, D 83 and D 96) during an interval in which no measurements were taken; and one (D 142) during a period of daily readings (March, 1927). The last one has not done it again so far, and three of the other four did not persist, so that in these animals the loop healed again and remained in good condition. But D 83 kept on chewing it until it became so scarred and so hard, that no more measurements could be taken. Eventually the animal died of hemorrhage. The reason for this is not clear, because repeated examination shows that there is no anesthesia or analgesia in the loop. Finally, one animal (D 25), on being lifted from the cage, caught its claw under the loop, kicked and tore it. This last accident (1 out of 97 good loops) and the "spinal trauma" are avoidable. The latter occurs in general on the first measurement and only in young animals, so it can be avoided by using full grown animals. This is preferable to packing the animal so tightly in the box that it cannot move. These are the only complications I have seen, which can be directly or indirectly attributed to the loop method. I have never seen thrombosis of the carotid or inflammation of the loop in animals under observation, which Van Leersum (2) reported having seen in one animal, or in fact any ill effects on the general condition of the rabbit. I have measured the blood pressure for as long as 15 months, with almost daily readings (1), and I am still measuring one rabbit 18 months after the first reading.

The data to be discussed now have been arranged in tabular form. They represent routine observations only, and not the results of any experimental condition imposed upon the animal, excepting of course the actual procedure of measuring the blood pressure and also a period of inanition of a few days to which several animals were subjected early in the work. Only one animal (No. 48-9) showed any effects of inanition, and the variations observed were not the extremes of the total variation recorded (1). On the other hand several of the animals developed diseases of various kinds, or were found to be pregnant or were the victims of some accident. These appear in Table I under "Remarks" and will be discussed later on. The blood pressure of a total of 90 rabbits (including the first 63 reported before) on the 1st day of observation is seen in Table II. The figures represent the arithmetic mean of the first 10 consecutive readings. The difference

between this table and the first one published (1) is partly due to better statistical treatment (a more careful distribution of the means). Three rabbits appear between 70 and 80 mm. Hg and none beyond 150 mm. The table, as it stands now, shows that on the 1st day of the examination, 60 per cent of the rabbits had a systolic blood pressure between 100 and 129 mm. Hg, and 85 per cent between 90 and 139 mm. Hg. The table, small as it is, compares in a general way with similar compilations made on men and women on the 1st day of examination. Concerning the published data on the blood pressure of the rabbit (direct measurement) it will be enough to quote the following: Volkmann (3) gives 90 and 108 mm. Hg. Meyer (4), after compiling the literature up to 1881, 51 experiments, comes to the following conclusion: "Dannach kann man als Grenzwerthe des mittleren Blutdrucks normalen Kaninchen ca. 70 und 140 mm. Hg annehmen." A remarkable conclusion, I think. Tigerstedt (5) says: "Beim Hunde schätzt man den Blutdruck auf 130–180 mm. Hg, beim Kaninchen auf 80–120."

The pulse rate of the rabbits reported in the present paper, counted on the loop itself at the end of each set of blood pressure readings, is shown in Table III. The pulse rate of the 1st day only has been given. By counting every other beat in a quarter of a minute, and multiplying by 8, it was possible to obtain the fast rates recorded. The error in these figures is at most ± 8, small for the purpose. It appears that the pulse rate of 84.7 per cent of these rabbits ranged, during the first measurement, between 180 and 259 beats per minute. Tigerstedt (5) quotes the figures of Colin (1888), 120–150 per minute, and Ellinger (1894), 120–160 per minute, as the pulse rate of the rabbit. I have had no access to the original works quoted by Tigerstedt, so I do not know under what conditions Colin and Ellinger counted the pulse rate. But since it is much simpler to count the pulse than to measure the blood pressure, and since it can be done with very little disturbance to the animal, the pulse rate becomes an invaluable index of the presence of psychic or other factors which may have at the same time some effect on the blood pressure.

It is important to note that neither the mean of blood pressure nor the pulse rate of the *first* observation is in general the highest recorded during the whole period. Thus, for instance, only five

showed on the 1st day as fast a pulse rate as the fastest during the entire interval of observation of the respective animals (D 12, D 21, D 87, D 88, 48-7), and of these only one was near the maximum of the whole series (No. 48-7, 296 per minute), discarding of course those animals in which only one observation was made. With regard to the blood pressure, the number of animals in which the highest single reading of the corresponding period of observation was at most 10 mm. Hg higher than the mean of the 1st day, was sixteen, and of these eight were examined during less than 1 week. The remaining eight are D 10, 12, 21, 24, 34, 41, 116, 142. The distribution of the

TABLE IV.

Blood pressure	No. animals	Per cent
mm. Hg		
200–	1	1.2
170–179	4	4.9
160–169	7	8.5
150–159	10	12.2
140–149	15	18.3
130–139	22	26.8
120–129	12	14.6
110–119	7	8.5
100–109	2	2.5
90–99	0	
80–89	2	2.5
	82	100.0

maximum blood pressure by animals is seen in Table IV. By maximum is meant the highest blood pressure recorded *at least once* during the whole period of observation of the corresponding animal. Eight animals have been omitted because they were examined only during 1, 2 or 3 days. The table shows that on prolonging the observation period, 71.9 per cent reached 130 mm. Hg, 45.1 per cent reached 140, and 26.8 per cent reached 150, at least once, whereas on the first examination only 22.2 per cent were at or above 130 mm. Hg (see Table II).

I shall consider in more detail those which reached or passed 160 mm. Hg. D 8, D 19, D 21, D 65 and D 80 had only isolated high

figures, so that in their protocols there is no *mean* of 10 readings at or above 160. Nos. 37-2, 48-7 and D 94 had only one *mean*, and Nos. 39-7 and D 22 two *means* above 160 mm. Hg. D 69 showed *means* between 160 and 173, 11 times out of 76 (14 per cent), *scattered* throughout the period of observation. There remains one rabbit, No. 48-9, whose graph was reproduced in the first report (1). The number of *means* at or above 160 is 92, out of 516 (18 per cent), but what makes this observation noteworthy is the fact that, excepting scattered high means in October and December, 1923, practically

TABLE V.

Blood pressure	No. daily averages		Per cent	
	No. 48-3	No. 48-9	No. 48-3	No. 48-9
mm. Hg				
190–199		1		0.3
180–189		3		1.0
170–179		15		5.0
160–169		33		11.0
150–159		76		25.2
140–149		84		27.9
130–139	6	57	2.2	18.9
120–129	12	31	4.5	10.3
110–119	34	1	12.7	0.3
100–109	117		43.8	
90–99	79		29.6	
80–89	19		7.1	
Total.....	267	301		

all the readings made in September and the first third of October, 1924, were at or above 160 mm. Hg.

What is the behavior of the pulse rate? In general, when the blood pressure rises in a normal rabbit, the pulse rate increases, but the converse is not true: an increase in pulse rate is not necessarily acompanied by a rise in blood pressure. If the animal resists, whether the blood pressure rises or not, the pulse rate increases. In Table I the exceptional animals from the point of view of resistance are referred to by the word "resistance" under "Remarks." Under conditions of "excitement" there may be no effect on pulse rate or

blood pressure, or a marked increase in both or only in the pulse rate. Excitement is an unsatisfactory word to use, because it involves so much which is subjective. Without attempting to define it, I shall give a list of the observations included under this heading: tickling the animal's nose or closing its nostrils for a few seconds during a set of measurements; after a few days of inanition putting a piece of carrot under its nose, while measurements are being taken; placing adult male rabbits, after several months confinement, with females, both in heat and not in heat, and taking the blood pressure and pulse rate before and after copulation. Under these conditions "excitement" is expected to be produced, and the animals show, indeed, signs which can be interpreted as such. Yet the results on blood pressure or pulse rate are not consistent or uniform in all the rabbits examined. A few examples may be seen in the first paper (1). The meaning of "an increase in pulse rate" will be understood from an examination of the figures of the two animals which exhibited the highest blood pressure, D 69 and No. 48-9.

TABLE VI.

Pulse rate	Daily averages		Per cent	
	No. 48-3	No. 48-9	No. 48-3	No. 48-9
280–299		2		0.7
260–279	1	12	0.3	4.0
240–259		59	0.0	19.7
220–239	8	77	3.0	25.8
200–219	25	94	9.5	31.4
180–199	42	41	16.0	13.7
160–179	110	12	41.7	4.0
140–159	57	2	21.6	0.7
120–139	20		7.6	
100–119	1		0.3	
Total.....	264	299		

D 69, as stated above, showed 11 means between 160.2 and 173 mm. Hg (with a mean of the means of 165) and the pulse rate of the corresponding days was between 208 and 248 per minute (mean 229). No. 48-9 showed 29 consecutive means between 155 and 193 mm.

Hg, with a mean of the means of 174, and the pulse rate of the corresponding days varied between 212 and 272 (mean 245).

The totals of the data from Nos. 48-9 and 48-3 are seen in Tables V and VI. Their graphs may be seen in the first paper (1). They represent the limiting cases found in this investigation. A few animals had a lower blood pressure than No. 48-3, but they were examined for a much shorter time, or their pulse rate was higher, or their figures were more scattered in distribution, or their behavior was not quite so good. Again, no other animal had a higher blood pressure than No. 48-9. So that these observations help in determining what might be called the boundary conditions of experimental hypertension in rabbits.

It is not practicable to present all the data in detail. If ever a compilation on the "normal" rabbit were attempted (and such a compilation is an urgent need of laboratory workers),[1] then this material would find its proper place there. It is only necessary here to show the salient facts observed on which a criterion for a pathological rise in blood pressure in the rabbit may rest. It must be borne in mind, that by "normal blood pressure of the rabbit" is meant the systolic blood pressure of a healthy looking rabbit *under the conditions of the measurement.* The practice of taking the blood pressure of the rabbit a few days before the experiment, *as control,* has less value than is generally conceded. 1 week, or 1 month of observation, is not sufficient to characterize completely the blood pressure curve of a rabbit, and does not guarantee that the pressure will behave in the same way in a subsequent interval of time. Finally, the purpose of the investigation must be considered. For acute experiments, there is little need of a carotid loop. On the other hand, a method like this is indicated for the study of prolonged alterations in blood pressure, which may simulate either the clinical picture of hypertension or Addison's syndrome. With these reservations in mind, a criterion could be formulated as follows:

(*a*) Under the conditions of the measurement, a rabbit may be said to have a pathologically high blood pressure, if the blood pressure

[1] The recent work of Brown, Pearce and Van Allen is an excellent beginning in this direction (11).

oscillates *above 180 mm. Hg* and does not fall below that figure during a length of time dependent on the nature of the experiment.

(*b*) Under the same conditions, the blood pressure may be considered pathologically high if it oscillates *about 170 mm. Hg* with a concomitant pulse rate *below 200 beats per minute.*

The introduction of the pulse rate in (*b*) allows the avoidance of consideration of resistance, "excitement," and the like, and makes unnecessary the discarding of any figure in the course of the investigation. It seems superfluous to add that these criteria are provisional. Strictly speaking they rest on observations made on 90 "normal" animals (over 30,000 blood pressure readings, about 2,900 pulse rate counts), but they are reinforced by the subsequent experience with these animals under several pathological conditions. Since these observations, moreover, spread over 4 years, and since the animals were obtained from different dealers in different years, it is expected that future experience with the method here adopted will not differ grossly from that described in the present report. The observations of Van Leersum himself (1911, 1912) on twelve rabbits, agree very well with mine.

An Important Source of Error of the Method.—The following phenomenon has been observed in several rabbits.

D 138, Feb. 4, 1926, 3.09 p.m.* 100–101–79–82–84 = 93–98–98–100 = 103–97–100–99 = 102–101–103–103 = 103–92–92 (pulse rate 232).

D 80, Jan. 21, 1925, 10.55 a.m.* 152–102–112–108–112–118–126 = 141–132–132–136 = 146–143–143–145 = 145–143–142 = 141–142 (pulse rate 224).

Jan. 30, 1925, 11.36 a.m.* 144–140–140–138 = 139–139–140–105 = 102–108–116–120 = 130–131–130–135 = 130–132–128–132 (pulse rate 224).

* Cuff adjusted to loop.

This abrupt fall in blood pressure occurs at any time in a given rabbit. If observed once in an animal it may be observed many times. No sign of a more general character accompanies this phenomenon: no alteration in pulse rate, no change in behavior, no respiratory disturbance, no pupillary effect. It may probably be explained by a local constriction of the carotid under the influence of

the pressure applied on the cuff. The fact that it does not appear in all animals is not an objection to this explanation, because the carotid is subjected to this stimulus intermittently for a short time (3 to 5 minutes), and it is not to be expected that all carotids will react in the same way to a given stimulus. It is clear that the change must be local and not the expression of an actual fall of arterial pressure, which would be accompanied by a change of pulse rate. In Table I it appears under "Remarks" as "constriction." In some animals it is very slight, for instance:

D 78, Nov. 17, 1924, 3.18 p.m.* 138–132–133–132 = 132–130–96 = 129–128–127–127.

In others it is frequent and becomes disturbing, as in the following example:

D 6, Jan. 26, 1924, 10.27 a.m.* 115–110–104–89–88 = 112–113–115 = 114–112– (pulse rate 184).
114–112–113–97 = 106–112–106–108 = 112–116 (pulse rate 176).

*Cuff adjusted to loop.

I have discarded no animal on this account, but the "minimum blood pressure" has no meaning under these conditions, and has been omitted from the table. It is enough to recognize this phenomenon to avoid the error produced by it.

Changes in the Carotid within the Loop.—The majority of the carotids enclosed in a loop showed after the death of the animal a marked transverse striation of the intima. The striations are due to a slight thickening of the intima. Under the microscope the lesion is not well defined: a slight sclerosis of the subintimal tissues and here and there a moderate vacuolization of the muscular coat. This striation has been observed in the remaining parts of several carotids, a long time after the loop had been severed on account of necrosis; that is, in arteries to which *no cuff had ever been applied.* It has been found absent in arteries in which measurements had been taken for a considerable time. In a few animals which died immediately after the operation (possibly ether death), the examination of the carotid revealed sharp transverse lines of intimal tearing, very pr .bly produced by stretching the artery during dissection. Befor .his injury was recognized and avoided, it was not uncommon to see, before the final suture of the loop, small extravasations of blood on the muscular layer of the carotid, which gave to the artery a slightly beaded appear-

ance, the more noticeable since the artery at the end of the operation contracts down through exposure. With more care in the manipulation of the artery this lesion may be reduced in extent, and possibly eliminated altogether. The fact is, the rabbit's carotid is a very thin and delicate object and must be treated accordingly. I think that the lesion observed in a good number of the carotids is due to the cicatrization of these intimal operative injuries.

Shapiro and Seecof (7), reviewing some of the methods of blood pressure measurement in the rabbit, say that Van Leersum's would be an excellent one "provided the artery could be thus isolated without altering the compressibility of its walls through inflammatory reactions. This objection seems to render the method both impractical and prohibitive." The authors fail to realize that the method is not sensitive enough to detect small changes in the thickness of the artery. The cuff effect in this method consists of several parts, the cuff proper (a piece of cotton fabric and of rubber tubing), the skin with and without hair, subcutaneous tissue and the vessel wall. Of these, the first three become softer and more supple in time, which, theoretically at least, would lower the value of the total effect, whereas the thickening of the intima is not progressive as far as I have been able to ascertain. Under these conditions, fluctuations of 40 mm. Hg *in one animal* several days, or several weeks apart, or occasionally in 1 day, cannot be due to variation in compressibility of the arterial wall. Finally, in experiments on arteriosclerosis (to be reported soon) where *calcification* was found in the media of the carotid within the loop, as well as in other large vessels, and in the aorta, no constant elevation of the blood pressure was observed.

MacWilliam in his review (8) on the blood pressure of man says: "It may be taken as established that high blood pressure readings, when carefully taken, represent approximately correct measurements of the actual intra-arterial pressures as a rule. It is only in a small minority of abnormal cases of thickened arteries with excessive tonic contraction, etc., that serious discrepancy may occur, sclerotic conditions without muscular contraction having no important influence. Digital compression for 3 or 4 minutes or massage of the artery are useful in removing abnormal resistance and have the advantage of not causing congestion of the limb which may arise from repeated compressions by the armlet." The lesion in the carotid of my rabbits is of the type considered by MacWilliam as having no important influence, and the method, as actually used, amounts to a digital massage of the artery, so that, if this massage were as efficacious as MacWilliam believes and such "excessive tonic contraction" were ever present in the rabbit, the method would automatically eliminate this source of error.

Blood Pressure in Pathological Conditions.—After what has been said before it will be easy to see the difficulty of attaching importance

to small variations in blood pressure, within the range of figures between 80 and 170 mm. Hg. Their importance increases as the pressure approaches these limiting values. The diseases that have occurred in animals under observation (not experimented upon, excepting the blood pressure readings) are, in order of decreasing frequency: coryza, ear canker, pneumonia (with or without pleurisy), purulent pleurisy, subcutaneous abscesses, meningitis (drooping and rotation of head). Diseases found at autopsy, not diagnosed during life: coccidial cysts in abdomen, otitis media (one or both sides, without meningeal involvement), scarred kidneys, arteriosclerosis of aorta (slight), pulmonary abscesses (discrete), pulmonary mycosis, mediastinal abscess. Accidental injuries: "spinal trauma," "sciatic injury," total infarction of kidney (ligature of renal vein during adrenalectomy).

Of all these conditions only two deserve special mention: "Spinal trauma" and meningitis. During the terminal coma of the latter the blood pressure is low, 70–80 mm. Hg. Occasionally, however, the pulse is slow and irregular, and the actual blood pressure readings are not reliable. Concerning "trauma," the observation is as follows: the animal is within the box, quiet; blood pressure is taken as usual. Suddenly the animal moves within. The motion may not appear to be of excessive violence. The blood pressure rises abruptly (140–150 mm. Hg) and the pulse is very slow and strong. Soon afterwards (1 minute or so) the pressure gradually falls and the pulse rate increases. Greater details cannot be given because the occurrence fortunately is rare and takes place within a very short time.

In the other conditions the blood pressure findings are discordant. In one rabbit suffering from a large unnoticed abscess of the thigh, the blood pressure was low (the graph has been reproduced in another paper (9)). During the course of pleuropneumonias the blood pressure may lie between 80 and 90 (see graph for No. 47–0 (10)), or between 90 and 110 mm. Hg. In No. 48-7 the mediastinal abscess, a large mass twice as large as the heart, situated on the right side, anteriorly, and adherent to the pericardium, showed a certain effect on the blood pressure, the more interesting since it was completely misunderstood during life. The observation is so unusual, that an abstract of the protocol is presented.

No. 48-7, albino, male rabbit. July 31, 1923, carotid loop is made, weight 2.440 kilos. Partial necrosis of distal portion of loop. Healed completely.

Aug. 15, first blood pressure readings (see Table I). Aug. 18– Sept. 8, blood pressure between 120 and 129 mm. Hg (74 per cent of daily means). Oct. 6 and Dec. 2, 1923, blood pressure between 130 and 149 (72.5 per cent of daily means.)

Dec. 3, lead carbonate smeared in carrot, fed by hand, about 25 mg. daily. Dec. 8, weight 2.550 kilos; Dec. 23, best weight, 2.675 kilos. Dec. 24, daily dose of lead carbonate increased to 78 mg.

Jan. 4, 1924, diminished appetite; Jan. 12, weight 2.480 kilos; Jan. 17, weight 2.190 kilos. Jan. 20, little appetite. Jan. 26, lowering of blood pressure; blood pressure from Dec. 3, 1923, to Jan. 24, 1924, between 120 and 139 (78.4 per cent of daily means). Feb. 6, lowest weight, 2.120 kilos.

The loss in weight and the lowering of blood pressure (see below) were thought to be due to lead carbonate, and the latter was interrupted until Feb. 18, 1924. From Feb. 18 to May 31, lead was given daily in increasing dose, from 40 up to 300 mg. Feb. 16, weight 2.260 kilos. Weight increases slowly in spite of increasing dose of lead.

Feb. 20, symptoms while drinking water, as if water had entered the trachea. Abundant râles. Bronchial (or tracheal) moisture persisted until Mar. 19. On Mar. 1, it was thought that the animal had a foreign body in the trachea. Apr. 11, weight 2.480 kilos; May 26, weight 2.285 kilos. June 2, animal looks sick. Blood pressure from Jan. 26 to May 31, 1924, between 100 and 119 mm. Hg (87 per cent of daily means). Concerning the pulse rate the only thing at all remarkable is the fast rate in the second half of May, between 240 and 280 per minute (mean 258). Blood pressure on June 2, between 74 and 78 mm. Hg, pulse rate 208. June 3, lies on side, does not move. June 4, dead.

The beginning of this disease may have been concomitant with the first fall in weight in the latter part of Dec., 1923, and the formation of a mass in the mediastinum (probably from lymph nodes) may account for the spell on Feb. 20 and the slow recovery from it. The increase in weight in spite of increasing dose of lead carbonate militates strongly against lead poisoning. Lead given in this fashion to a group of animals (of which No. 48-7 is one) has shown no deleterious action (experiments to be reported later). The animal was in excellent physical condition up to June 2, 1924. It had been mated repeatedly during the whole observation, the last copulation having occurred on May 20.

Carotid Loop in Dogs.—As a further check on the method, loops were made in two dogs. Here it was possible to use the stethoscope and find auscultatory criteria for systolic and diastolic pressure similar to those used in man. The daily means of one of the dogs ranged from 123 to 165 mm. Hg, 77.7 per cent lying between 130 and 149 (85 days), for the systolic pressure, and from 69.1 to 103 mm. Hg, 87 per cent between 80 and 99, for the diastolic. The pulse rate oscil-

lated between about 60 and 184 per minute, 87 per cent of the counts being between 80 and 139. The other dog was examined during a much shorter time: systolic between 125.8 and 164.5 mm. Hg (10 daily means), diastolic 80–103, pulse rate 104–160. Both these dogs were subjected later on to double adrenalectomy. A complete report will appear shortly in collaboration with Dr. J. M. Rogoff.

SUMMARY.

The blood pressure and pulse rate of 90 normal rabbits have been studied for various periods of time, from 1 day (accidental death interrupting the observation) to 15 months. The main data are presented in a table containing the blood pressure and pulse rate on the 1st day of observation, the maximum and minimum of both during the entire period of observation of each animal, together with the sex and weight of the animal. Separate tables are given showing the distribution of blood pressure, pulse rate and the "maximum" blood pressure by animals. Detailed data on two animals observed for the longest time are given in tabular form. The anatomical changes that occur in some carotids enclosed in a loop are described and discussed. Considerations on "excitement" and pathological conditions which arise spontaneously in rabbits are given. A criterion for a pathologically high blood pressure in rabbits is proposed.

The blood pressure of the normal rabbit ranges between 70 and 170 mm. Hg. The pulse rate, taken simultaneously with the blood pressure, fluctuates between 112 and 300 per minute.

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EXPERIMENTAL ATHEROSCLEROSIS AND BLOOD PRESSURE IN THE RABBIT.

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The relation between atherosclerosis and blood pressure has engaged the attention of several workers following the statement of Fahr (1) and Van Leersum (2) that rabbits fed on abnormal diet (egg, liver) develop a high blood pressure. The published material that I have examined is disconcerting, due not so much to the variety of methods used to determine the blood pressure, as to the lack of sufficient information concerning the "normal" fluctuations of blood pressure, that is, the fluctuations observed in normal animals with the particular method used. Van Leersum, who claimed to have found a marked elevation of blood pressure under the influence of a liver diet, obtained no lesion whatever in the circulatory apparatus of his rabbits. Again most workers seem to be under the impression that the blood pressure is a constant, that is, that if the blood pressure oscillates in a certain region for a few weeks before the experiment, then any increase above this region that may occur afterwards during any experimental condition is necessarily due to the experimental condition, although these values may be well within values recorded from other normal animals. I have had considerable experience with Van Leersum's method (3, 4) and have given curves which sufficiently illustrate the fallacy of that assumption. For instance, in the graph of Rabbit 48-3 (3) it may be seen that the blood pressure oscillated around 100 mm. Hg for fully 10 months (from November, 1923, to September, 1924) and then rose in September and October, reaching 140 (average) on September 27. If an experiment had been started in the last week of August and this rise had been observed, the observation would have been supported by a good control period, but an inaccurate conclusion could have been drawn. Another type of curve is shown here, and

still others may be found in former papers (3–5). To avoid repetition, by "blood pressure" and "normal range" is understood the systolic blood pressure of the rabbit as obtained from a carotid loop (Van Leersum's method) and the range of blood pressure in normal rabbits as determined previously by the writer.

The experimental atherosclerosis of the rabbit has in itself considerable intrinsic interest, so it seemed worth while to repeat the experiment. The literature on cholesterol feeding experiments is voluminous. For an introduction to the subject the reader is referred to the references given here, particularly Schönheimer. In the present work egg yolk was chosen since natural emulsions have been found best suited for the purpose. The rabbits (five in number) were given their usual food (hay, oats, greens) throughout the experiment; the yolk of one or two eggs was mixed with powdered unleavened bread and dried at 37°C., the whole appearing finally as yellowish crisp masses. The animals ate it readily in the beginning, but after some time they seemed to tire of it, so the yolk was diluted with plain water and the stomach tube used. The blood pressure was taken daily in the manner explained elsewhere (3). These five animals were chosen at random. One had been measured as a routine for several months, others for less time. Two of the five received lead carbonate by mouth in additon to the egg yolk. It seems justifiable to report the two cases where lead was also given, D 1, D 10, for the following reasons:

1. The association of lead poisoning and high blood pressure in man has always been considered in clinical medicine.

2. Lead carbonate in the form given to these animals and lead acetate given by stomach tube, in my experience, do not produce high blood pressure in the rabbit (unpublished data).

3. Recent work done in this country on the general subject of lead poisoning throws doubt on the efficiency of absorption of lead by the gastrointestinal canal (summarized in Reference 6).

4. There is no essential fact in the behavior or in the autopsy of these two animals that could be attributed with certainty to lead.

The curves were plotted at the completion of the experiment. The organs of the animals were carefully examined after death. Microscopic examination, however, was not systematically done. A brief analysis of Van Leersum's report (2) will be found at the end of the

paper, followed by a note on the results obtained by other methods of measuring the blood pressure.

The essential data on these five animals will be given in the form of condensed protocols.

D 1.—Male, brown, Belgian rabbit. Nov. 30, 1923, carotid loop is made; weight 2.310 kilos. Jan. 7, 1924, blood pressure measurements started; weight 2.625 kilos.

Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos" and 30 mg. of lead carbonate smeared in carrot and fed by hand, daily; weight 2.855 kilos. Mar. 13, daily dose of lead carbonate increased to 60 mg. Mar. 29, daily dose of lead carbonate increased to 80 mg. Apr. 6, best weight 3.360 kilos.

Apr. 14, conjugate motions of eyes and head, toward the right, with drooping of right ear. Lead and egg feeding are interrupted. Apr. 16, same condition; weight 2.660 kilos. Apr. 21, right ear is full of a foul smelling creamy pus; weight 2.410 kilos. Apr. 30, eyes were found to possess well developed corneal arcs. May 11, weight 2.395 kilos. May 17, death in coma.

Total number of yolks consumed, 40. Total amount of lead carbonate given, 2.690 gm.

Blood Pressure.—Highest pressure recorded was 135 mm. Hg on February 7. This animal was one of those which exhibit a phenomenon described in the preceding paper (4) and ascribed to a local constriction of the carotid under the stimulation of the external pressure applied on the cuff. It is well illustrated by the following examples.

Feb. 6, 1924, 10.21 a.m.* 121–71–101–112 = 115–126–125–128 = 125–125–120–123 = 128–124–122–120 = 121–122–124–127 (pulse rate 192).

Mar. 4, 1924, 10.09 a.m.* 103–88–68–0 (15 seconds)–99–92–93–93 = 96–95–93–95 = 76–69–70–89 = 95–93–94–91 = 92–91–97–95 = 95–95–96–93 (pulse rate 168).

* Cuff adjusted to loop.

This phenomenon renders the tabulation or plotting of the figures almost impossible. It appeared throughout the course of the experiment, but not every day. It was absent in the last part of the experiment, when the intracranial complication of the otitis media became evident. The blood pressure during this latter period was, in general, low, oscillating between 70 and 90. The pulse rate varied between

136 (February 23 and April 23) and 220 (January 17) per minute. During the terminal coma the pulse became very irregular, a few beats passing through at 90 mm. Hg.

Autopsy.—Heart: base of large mitral cusp infiltrated with fatty substances. Aorta: large patch of infiltration at opening of arch branches, extending for a short distance into the common root of carotids and into left subclavian. In ascending arch there are a few minute nodular elevations. Nothing in thoracic aorta. In abdominal aorta, several small nodules and two streaks, one at root of celiac trunk, another at level of renal arteries. These two streaks are perpendicular to the axis of the aorta. Remainder of aorta and iliacs, normal. Pulmonary artery shows several elongated patches of moderate size, along posterior wall, parallel to the axis. Left adrenal, 950 mg.; right 860 mg. (weighed on March 26, 1927, in formol in the interval, see discussion). Corneal arcs, bilateral, well formed. Brain: white, thick, purulent exudate at level of tentorium cerebelli, both sides of midline. Right middle ear is filled with a creamy pus.

D 10.—Female, brown rabbit. Dec. 6, 1923, left carotid loop is made; weight 2.280 kilos. Jan. 7, 1924, blood pressure readings started; weight 2.465 kilos. Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos" and 30 mg. lead carbonate smeared in carrot and fed by hand, daily; weight 2.705 kilos. Mar. 7 and 12, best weight 2.840 kilos. Mar. 13, daily dose of lead carbonate increased to 60 mg. Mar. 26, animal looks sick; weak; egg and lead are withheld. Mar. 27, weight 2.555 kilos. Apr. 16, egg given again; animal has recovered its former appearance and behavior, but not its weight; weight 2.515 kilos. Apr. 26, weight 2.700 kilos.

May 1, egg given through stomach tube; lead carbonate given again, 50 mg. daily. May 2, no corneal arc in either eye. May 15–17, lead carbonate suspended in egg yolk emulsion, stomach tube. May 16, weight 2.350 kilos. May 18, dead.

Total number of egg yolks consumed, 55. Total amount of lead carbonate given, 1.780 gm.

Blood Pressure.—Highest figures recorded before experiment: 146 (January 7, 1924), 150 (January 23), 149 (February 25) with averages for day, 140.4, 140.3, 144.8 respectively. Highest figures recorded during experiment: 152 (March 5), 150 (March 6), 149 (March 11), 148 (March 12), 150 (March 15) with averages for day, 139.6 (30 readings), 143.2, 145.9, 140.6, 143.0 respectively. From March 26 to April 10, the blood pressure reached the lowest level observed during

the whole experiment, as low as 87 mm. Hg. This is the same period in which the animal appeared sick, concomitantly with loss in weight, loss in appetite, and, as it will be seen afterwards, increase in the pulse rate. I have no explanation for this. I have seen nothing like it in my experiments with egg yolk alone, or in the other animal which received lead together with the egg, or in several animals which have had lead carbonate or lead acetate alone (unpublished data). The pulse rate during this period oscillated between 216 and 288 per minute. The contrast in the behavior of pulse rate and blood pressure in the three periods, before March 26, from March 26 to April 10, and after April 10, is best seen in tabular form, where I have taken figures corresponding to the two extremes and mean of pulse rate for the respective periods.

Date	Pulse rate per min.	Blood pressure
1924		*mm. Hg*
Jan. 7	264*	138–146
Feb. 5	264	119–130
Feb. 9	272	118–124
Feb. 25	280	139–149
Jan. 9	232	116–138
Jan. 22	240	110–130
Mar. 17	232	130–135
Feb. 11	188	101–116
Mar. 26	216	87–92
Mar. 31	264	98–107
Apr. 5	280	97–101
Apr. 7	288	93–103
Apr. 16	192	102–110
May 1	144	111–126
May 14	240	122–129

* These fast rates are counted by groups of two $\frac{\downarrow\downarrow}{1}$ $\frac{\downarrow\downarrow}{2}$ $\frac{\downarrow\downarrow}{3}$.

With increasing rates the pulse becomes very rhythmic and this process of computation is accordingly easier.

Autopsy.—Aorta is mottled throughout with yellow spots and streaks, slightly elevated, parallel to the axis of the vessel; somewhat more abundant in arch and thoracic portions. For a distance of 1.5

cm. above the opening of the celiac trunk these small infiltrated areas become confluent. Infiltration from this point downward is less and less marked. Pulmonary artery shows a large, irregular, slightly raised patch, at bifurcation, extending both ways for a short distance. In left kidney there are a few yellow streaks in outer zone of pyramid. Eyes show no visible corneal arcs. Adrenals are large, right weighs 440 mg. (weighed on March 26, 1927, almost 3 years in formaldehyde solution; left adrenal has been split open and a piece of central portion cut off for microscopic examination; there was no obvious difference in size at the time of the autopsy).

D 6.—Female, brown rabbit. Dec. 4, 1923, carotid loop is made; weight 1.785 kilos. Jan. 7, 1924, first blood pressure readings; weight 2.065 kilos.

Feb. 26, feeding experiment began; one egg yolk mixed with powdered "Matzos," daily; weight 2.495 kilos. Apr. 3rd and 4th weeks, rut. Apr. 26, best weight 2.820 kilos. Apr. 30, egg yolk given by stomach tube.

May 2, corneal arc is well formed in right eye, spreading toward center of cornea for a distance of 3 mm. from limbus. In left eye there begins to appear a faint, delicate, white line next to iridocorneal junction. June 28, weight 2.340 kilos.

July 14, two egg yolks by stomach tube, daily. Aug. 19, weight 2.165 kilos. Aug. 21, animal is cold, wabbly, looks sick. Egg feeding is discontinued. Aug. 22, very weak, cold. Died at 11.30 p.m.

Total number of egg yolks consumed, 210 (without interruption, except isolated days).

Blood Pressure.—Range of figures where highest values were obtained, (*a*) before egg yolk feeding, (*b*) during egg yolk feeding, together with mean of set and pulse rate:

Date	Oscillation	Mean	No. readings	Pulse rate
1924	*mm. Hg*	*mm. Hg*		
(*a*) Jan. 14	128–134	130.4	10	168
Jan. 15	129–134	130.4	10	160
Feb. 26	128–140	133.7	10	
(*b*) Mar. 6	124–139	131.4	20	176
Mar. 27	105–135	115.2	20	160
Apr. 10	118–138	128.6	20	216
Apr. 29	122–143	136.1	19	216
May 3	114–142	134.2	20	216
May 6	120–141	130.9	20	200

Toward the end of May, during the whole of June and first half of July, the pressure reached the lowest values observed during the entire experiment, oscillating between 72 and 108 mm. Hg. Pressure above 120 was recorded on May 17, and the next pressure above 120 was recorded on July 18. The lowest temperature of the room where the measurements were done, was, in this interval of time, 19°C. (May 22, 11.05 a.m.), blood pressure 86–109, mean 97.8 (20 readings), pulse rate 152 per minute. The highest temperature in the same interval, 30°C. (June 24, 3.53 p.m.), blood pressure 85–93, mean 90.1 (10 readings), pulse rate 184 per minute. The thermometer is mounted on the stand of the manometer and the temperature read systematically at the end of the measurements. From July 18 on, the pressure was not as low as in the interval just discussed but reached levels in general not as high as those recorded before May 17, the only exception occurring on August 9, when the pressure rose to 141 after the rabbit moved during the measurement. The fastest pulse rate was 232 per minute, on February 19 and March 12, which did not coincide with the highest pressures. The lowest rate was 128 per minute, on June 28, with a blood pressure between 80 and 86, mean 83.1 (10 readings), and on August 22, when the rabbit was profoundly asthenic and the blood pressure was between 82 mm. and 90 mm. Hg (measured while animal was lying on its side).

Autopsy.—Marked dilatation of heart. Marked atherosis of aorta at root, arch and first portion of thoracic aorta, where intima is thoroughly infiltrated; from here on infiltration is patchy, mainly at the opening of branches, with a rather large patch at the opening of celiac trunk. The infiltration is greatest just beyond the opening of branches, in many instances forming like a crescent on the caudal side of the opening. Lumbar aorta is practically free; iliacs free. Carotids not involved, except at their opening, and excepting a small portion of the root of left carotid (that within loop), in continuation with the aortic infiltration. Aortic leaflets and large mitral cusp are slightly infiltrated. Pulmonary shows a large patch at bifurcation, extending both ways for a short distance. Root of pulmonary is completely free. Profound infiltration of liver, which feels hard, and in many areas is coarse, the capsule in these places being thick and opaque. Spleen large and pale. Adrenals are large; weighed on March 26, 1927

(after almost 3 years in formaldehyde solution), left 550 mg., right 500 mg. Ovaries thoroughly infiltrated, but scarcely larger than normal. Gall bladder much distended, walls not thickened. Bile is very thick. Lungs contain many whitish nodules throughout, not unlike a miliary tuberculosis, and small areas of congestion at both bases. The microscope revealed the presence of a radiating fungus in these nodules.

No. 47-0.—Male, gray, Belgian rabbit. July 7, 1923, left carotid loop is made; weight 2 kilos. July 31, first blood pressure readings. Jan. 12, 1924, weight 2.895 kilos.

Jan. 16, feeding experiment started; one egg yolk mixed with powdered "Matzos," daily. Feb. 16, best weight 3.360 kilos.

Apr. 30, well developed corneal arcs on upper segment of both corneæ. Egg yolk by stomach tube. May 2, corneal arcs spread to lower segments, equatorial parts remaining free. May 11, weight 2.645 kilos. May 12, looks sick. May 15, dead.

Total number of egg yolks consumed, 117 (without interruption).

Concerning the *blood pressure*, see Fig. 1 and discussion.

Autopsy.—Aorta thickened and opaque, intima greatly thickened, yellowish, rough and totally infiltrated from arch down to bifurcation; only spot free from atherosis is the root of the aorta where there is only a small elevated nodule. Pulmonary artery is almost as much involved as the aorta. Root of carotids and iliacs infiltrated. Small amount of fluid in abdomen. Walls of descending and transverse colon are yellowish. Spleen much enlarged, rounded edges, tough. Liver very pale, mottled with yellow. Adrenals very large (left 1.383 gm.; right 1.169 gm. weighed fresh), float in formol solution (10 per cent). Kidneys of normal size and consistency, surface smooth, yellow striations in pyramid following the normal rays; here and there, at base of pyramid, small yellowish nodules. Bilateral purulent pleurisy. Discrete patches of consolidation in left lung. Frozen sections of aorta show the intima to be *twice* as thick as the muscular coat. Histological details are omitted because they add nothing to the present knowledge of this experimental condition.

D 12.—Female, white rabbit. Dec. 10, 1923, left carotid loop is made; weight 1.855 kilos. Jan. 7, 1924, first blood pressure readings; weight 1.970 kilos.

Feb. 26, egg yolk feeding is started; weight 2.435 kilos (one egg yolk in bread, daily).

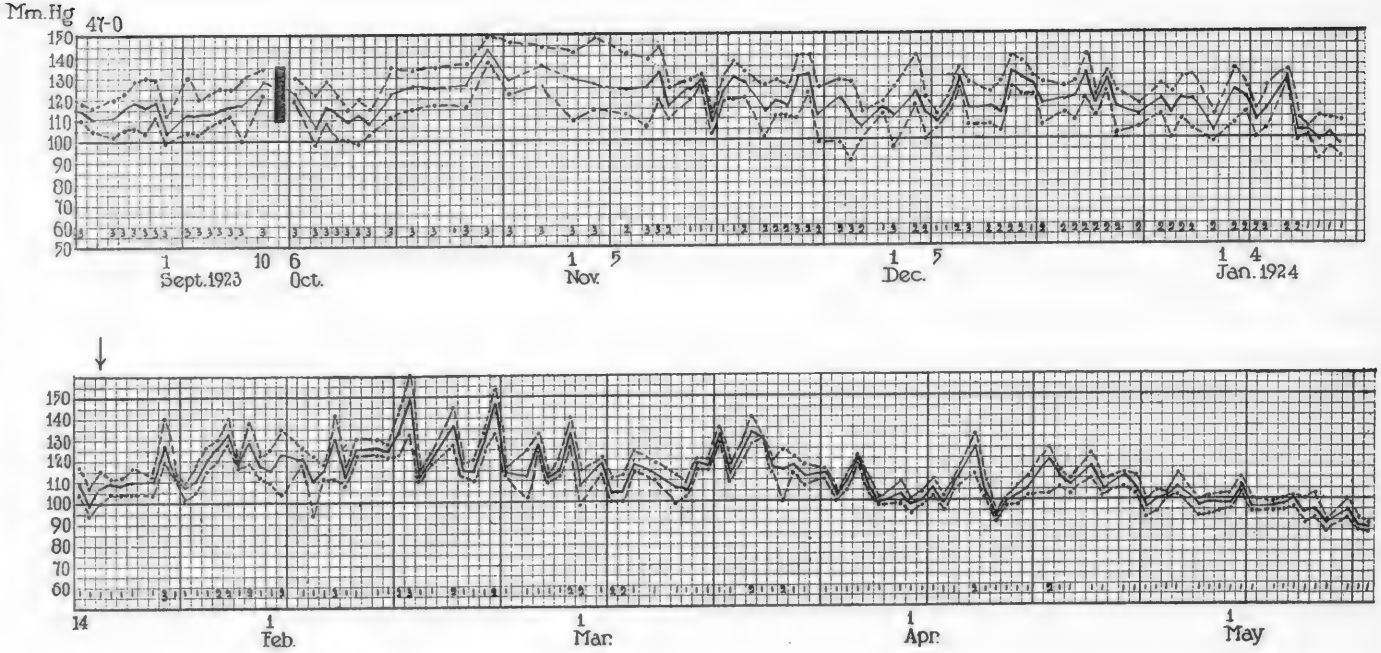
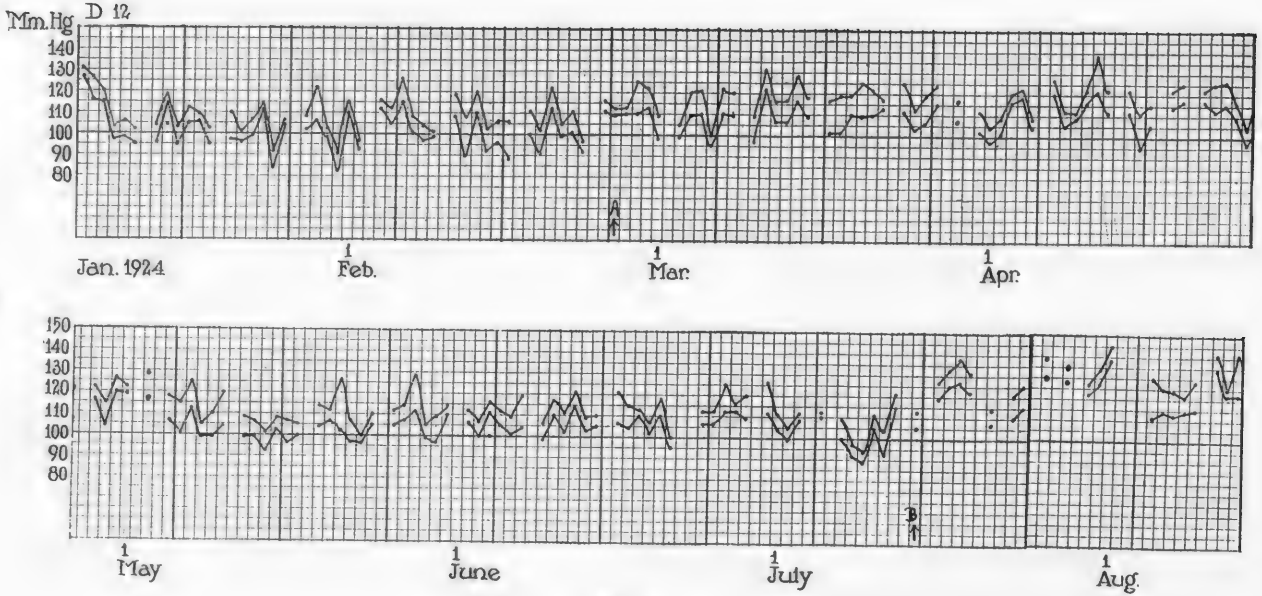

Mm.Hg
47-0
150
140
130
120
110
100
90
80
70
60
50
1
Sept. 1923
10
6
Oct.
1
Nov.
5
1
Dec.
5
1
Jan. 1924
4
14
1
Feb.
1
Mar.
1
Apr.
1
May

FIG. 1.


Mm.Hg
D 12
140
130
120
110
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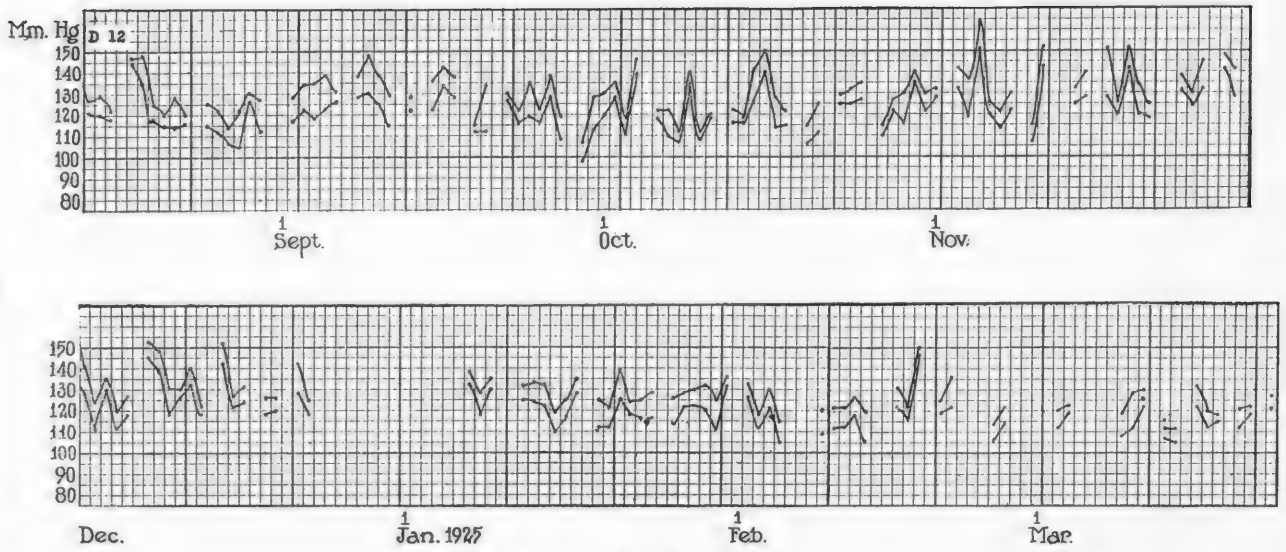
90
80
Jan. 1924
1 Feb.
1 Mar.
1 Apr.
150
140
130
120
110
100
90
80
1 May
1 June
1 July
1 Aug.

FIG. 2.


Mm. Hg
D 12
150
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1 Sept.
1 Oct.
1 Nov.
150
140
130
120
110
100
90
80
Dec.
1 Jan. 1925
1 Feb.
1 Mar.

FIG. 3.

Apr. 30, egg yolk in milk, through stomach tube. May 1, egg yolk in water, stomach tube, daily. Weight 2.615 kilos.

July 14, two egg yolks in water given, daily. Dec. 27, weight 3.335 kilos. Mar. 17, 1925, weight 3.220 kilos.

Mar. 23, animal in excellent condition. It was sacrificed (chloroform) because the experiment was considered to have lasted sufficiently long. The animal did not receive any eggs from Dec. 28, 1924, to Jan. 5, 1925, inclusive. Occasional days scattered throughout the experiment were missed also.

Total number of egg yolks consumed, 531.

For *blood pressure* see discussion. Graph is reproduced in Figs. 2 and 3.

Autopsy.— *Aorta* and *pulmonary* artery are opaque, thick. Intima is rough, yellowish, wrinkled. In the pulmonary artery the infiltration is greatest around the bifurcation and extends into its branches. In the aorta the infiltration begins at the ring and extends clear down to the iliacs. All the main trunks are infiltrated a short distance from their root. The arch is dilated, and here the infiltration is greatest. Carotids and iliacs are not infiltrated. Mitral valve is thoroughly infiltrated; tricuspid only slightly. Liver: intense fatty infiltration. Costal margin has made a deep impression on convex surface and in this area the infiltration is greatest. Spleen somewhat enlarged. Transverse colon possesses a distinct yellowish discoloration of the mucosa. Uteri are enlarged, and vagina is greatly dilated (contains abundant mucus); cervices, full of clear, transparent polypi. Uterine mucosa is full of like polypi. Adrenals are large, left weighs 570 mg., right 471 mg., weighed fresh. Kidneys normal in size and shape; surface moderately pitted; cut surface shows a few well defined, large yellow nodules in the boundary zone, somewhat spindle-shaped, with the long axis parallel to the medullary rays; besides these large fatty deposits there are fine yellowish striæ in the medulla.

DISCUSSION.

Autopsy Findings.—All the animals developed varying degrees of atherosclerosis of the aorta; from a few flat clusters scattered here and there (D 1) to a total infiltration from root to bifurcation (No. 47-0 and D 12). The most severe lesion was found in No. 47-0 (117 egg yolks) where the fatty intima formed two-thirds of the total thickness of the aorta (7). D 6, after 210 egg yolks, and D 12, after 531 yolks, had a

less profound infiltration, although it extended to the whole length of the aorta in D 12. The least infiltration occurred in D 1, which received the smallest number of eggs (40 eggs). The pulmonary artery was involved in all, the most extensive infiltration occurring again in No. 47-0.

The largest corneal infiltration was seen in D 6, a brown female rabbit. Corneal arcs were absent in D 10 (55 eggs) and D 12 (531 eggs). The former was a brown female rabbit, the latter an albino, female also. Schönheimer (8) concludes, from his experiments, that males are more resistant than females as far as the production of corneal arcs is concerned, and that extensive arcs are obtained only in females after prolonged administration of cholesterol. In accordance with this, the most extensive corneal infiltration was present in a female, but another female had no corneal arcs after 531 eggs. Since this animal was the only albino of the group, and Schönheimer says nothing about the color of the rabbits, this observation should be emphasized. On the other hand, D 10 had no corneal arcs after 55 egg yolks, while D 1, a brown male, had good corneal arcs after 40 eggs.

The fatty infiltration of the liver was most intense in D 12, without any evidence of a cirrhotic process; but in D 6 the liver was firm and in some places distinctly coarse. The spleen was considerably enlarged only in one (No. 47-0). The kidneys showed fatty streaks in the pyramid in three (D 10, No. 47-0, D 12) and discrete clumps in the boundary zone (Bailey) in two (No. 47-0 and D 12).

The adrenals were of good size in all. Unfortunately some of the adrenals were not weighed fresh, but after several years standing in formaldehyde solution. To get an approximate idea as to the effect of such prolonged fixation on the weight, the adrenals of No. 47-0 and D 12, which had been weighed at the time of the autopsy, were weighed again, that is, after an almost equally long formol fixation, with the result that the left gland of No. 47-0 *gained* about 8 per cent (the right had been sectioned and a piece removed for microscopic examination) whereas the adrenals of D 12 *lost* about 4 per cent. If it be assumed that all the other adrenals *gained* 8 per cent (to assume the worst case) and this amount be subtracted from the recorded weight, an average of 667 mg. for the left and somewhat higher for the right is obtained,

far greater than the corresponding averages 238 and 221 mg. for fourteen normal rabbits above 2 kilos in body weight and greater than the averages 353 and 341 mg. for fourteen rabbits above 2 kilos in body weight, whose thyroid and parathyroids had been removed by Marine (Stewart and Rogoff (9)). In the extensive statistical work of Brown, Pearce and Van Allen (10) on 645 normal *male* rabbits, mainly from eastern Pennsylvania and the immediate vicinity of New York City, the mean combined weight of the adrenals is given as 0.383 gm. More details are unnecessary, since all these findings have been described before (8). My sole purpose is to show that the present animals actually had the now well recognized picture of experimental cholesteatosis, and that in particular the aorta showed from slight to extreme fatty intimal deposits. None of these aortas showed any gross calcification of the media. Schönheimer observed a marked calcification of the thoracic aorta in one of his animals, and says, very naively, that it is probably not due to the diet, but rather to the use of the stomach tube, which through repeated, short elevations of the blood pressure, may act like adrenalin injections. Although he had Schmidtmann's method (11) at hand, he did not use it to see if there was really an elevation of blood pressure, which if found, could still be ascribed to struggle or excitement, since the animals do not take the tube without resisting, and granting, of course, that adrenalin necrosis is mechanically produced and not due to a toxic action or something else.

The kidney deserves special mention. Bailey has found it more frequently affected than other investigators (Schönheimer). Bailey found the surface pitted in four out of nine animals egg-fed, or in six out of eight whose kidney showed gross cholesterol lesions. I find striæ and nodules also, but scars in the cortex only in one (D 12), a moderate scarring in fact, and although this animal received nine times as many eggs in less than five times as many days as Bailey's Rabbit 7, the xanthomatose lesions appear insignificant when compared with the extraordinary lesions illustrated in Bailey's paper (his Fig. 7, kidney of Rabbit 7). There were no scars in the kidneys of D1 and D10 which received lead carbonate. I am inclined to believe that these xanthomatose formations are secondary to a preexisting scarring of the cortex, a view considered by Bailey himself and by Schönheimer.

Blood Pressure.—Fig. 1 contains *all* the essential data from No. 47-0. The lower broken line represents the lowest reading of the corresponding day, the upper broken line the highest reading. The solid line is the calculated arithmetic mean. No figure has been discarded, but the measurements taken from July 31 to August 23 have been omitted to shorten the graph. The range of pressure in the omitted period covers from 90 mm. to 134 mm. Hg, with a mean of 110.0 (420 readings). The small figures at bottom of the graph multiplied by 10 give the number of readings of each day. No readings were taken from September 11 to October 5 (black bar in graph), or on isolated days (break in sequence of bottom figures). Egg feeding was started on January 16 (arrow in graph). The only difference in the blood pressure curve before and during the egg feeding consists in the wider range of daily oscillations in the former, a difference readily accounted for by the larger number of daily readings. Toward the end, when the animal was obviously sick (see protocol), the blood pressure was low. The blood pressure reached about 150 on two occasions, both before and during egg feeding. The details of these days are as follows:

Oct. 24, 1923, 10.41 a.m. 145–144–146–144–147–143–144–142–141–141 (pulse rate 200).

10.49 a.m. 149–145–143–144–141–143–145–142–143–146 (pulse rate 160).

10.58 a.m. 144–146–142–142–139–137–137–140–139–138 (pulse rate 160).

Nov. 3, 1923, 3.45 p.m. 129–127–126–126 = 127–126–130–130 = 130–130 (pulse rate 200).

3.51 p.m. 146–148–146 = †128–120–115–116 = 116–116–116 (pulse rate 184).

At the point indicated by † animal moved backward, in box.

3.58 p.m. 129–130–129–128 = 121–120–123–125 = 121–123 (pulse rate 176).

Feb. 14, 1924, 12.08 p.m. 160–160–155–153 = 156–150–152–150 = 150–148–147–152 = 152–147–146–142 = 149–152–150–145 (pulse rate 200). Mouth piece removed for a few seconds, cuff in place, animal did not move.

12.17 p.m. 145–142–135–132 = 139–140–149–140 = 144–146 (pulse rate 188).

4.59 p.m. 118–121–122–122 = 128–129–129–131 = 136–134 (pulse rate 184).

5.07 p.m. 155–153–152–150 = 145–144–144–142 = 143–144 (pulse rate 180).

Feb. 22, 1924, 3.18 p.m. 138–133–139–139 = 142–142–145–143 = 150–150–149–144 = 149–150–150–149 = 150–153–153 = 153 (pulse rate 176).

Pulse rate behaved as follows:

Fastest, Aug. 30, 1923, 248 per minute, with a blood pressure between 125 and 130 mm. Hg, mean 126.7 (first 10 readings).

Slowest, Nov. 27, 1923, 128 per minute, blood pressure 90–104, mean 95.3 (1st 10 readings).

Apr. 19, 1924, 128 per minute, blood pressure 102–110, mean 104.8 (10 readings).

Apr. 30, 120 per minute, blood pressure 94–102, mean 98.3 (10 readings).

May 1, 128 per minute, blood pressure 95–102, mean 98.2 (10 readings).

On May 12, 13 and 14, when the animal was suffering from a pleuropneumonia, the pulse rate was 192, 224, 192, respectively, and the blood pressure oscillated between 84 and 100 mm. Hg. The curve illustrates one of those rare animals which cover the whole range from 90 to 150 mm. Hg, that is, almost the totality of the normal fluctuations of blood pressure in the rabbit. Without special indication it would be impossible to pick out the egg feeding period. D 1 with a maximum pressure of 135 mm. Hg and D 6 with a maximum of 143, may be dismissed without further discussion. D 10 does not differ essentially from No. 47-0.

There remains D 12, whose graph is reproduced in Figs. 2 and 3. During January and February, 1924, up to *A* (see Fig. 2), blood pressure oscillated between 83 and 132 mm. Hg, with a mean of 104.9. From *A* to *B*, 139 days (animal receiving one egg yolk daily), the blood pressure oscillated between 88 and 138 mm. Hg, with a mean of 109.4, the oscillations being somewhat greater during March and April than during May and June. From *B* on to the end of the experiment (animal receiving two egg yolks daily), the blood pressure was in general higher and the oscillations became larger, between 98 and 165 mm. Hg, with a mean of 124.0. Pulse rate behaved as follows:

Fastest, Nov. 5, 1924 (resistance), 256 per minute, blood pressure 151–164 mm. Hg, mean 155 (20 readings).

Nov. 17, 256 per minute, blood pressure 128–151, mean 135.3 (20 readings).

Slowest, June 3, 1924, 132 per minute, slightly arythmic, blood pressure 100–107 mm. Hg, mean 102.1 (10 readings).

July 9, 1924, 136 per minute, blood pressure 89–94, mean 91.4 (10 readings).

The protocols of the days of highest blood pressure are as follows:

Nov. 5, 1924, resistance. 2.32 p.m. 164–164–162 = 160–158–157–155 = 154–154–153–151 = 152–152–151–151 = 153–152–153–153 = 152 (pulse rate 256).

Dec. 1, 1924, 10.25 a.m. 165–163–160–155 = 152–153–149–150 = 145–142–142–142 = 142–142–141–139 = 138–140–139–135 (pulse rate 200).

The curve from *B* on to the end is representative of the average blood pressure of a good number of the normal rabbits. It compares well also with the curve of D 46 (Fig. 6, in a previous paper (5)) before and after double adrenalectomy, but oscillates about a lower level than D 65 (Fig. 4, in the same paper (5)), also adrenalectomized. Taken alone, this part of the curve has, therefore, little interest. It gains interest, only when brought into relation with the first part of the curve, and particularly when it is remembered that from *B* on two eggs were given daily instead of one. But, interesting as it may be, is it significant? The answer may be split in two according to the meaning to be attached to the word "significant." If by "significant" is understood "fluctuations beyond the range of blood pressure of normal rabbits in general" the answer is immediate and negative, as appears clearly enough from my former work (3, 4) and from the preceding paragraphs, or from another glance at the normal curve of No. 47-0 (Fig. 1). If by "significant" is meant "fluctuations within the normal range, but high relatively to the fluctuations of the pressure in the same animal during a period of observation, more or less short, before the experiment," (and these constitute the large majority of the claims found in the literature), then the answer is very difficult, perhaps impossible. It was stated at the beginning of this article that there is a fallacy in ascribing any rise in blood pressure to the experiment which is being done, because sometimes rises of pressure

without known cause were seen in animals employed for observation for a long time. Moreover, D 12 is the only one of five animals in which such an effect is observed. From *A* to *B* the animal consumed 130 egg yolks and it is highly probable, judging from the other four experiments and from Bailey's experience, that at the end of that time there was already a good infiltration of the aorta. So that from the standpoint of the etiological relation of high blood pressure to the development of atherosclerosis, this observation is not a favorable one, and the other four are no better. Although the pulmonary artery is just as frequently and almost as severely involved as the aorta, I have not seen any report claiming an increased blood pressure in the pulmonary artery of these rabbits. Not knowing how to measure this pressure, I shall not discuss it, but clearly an examination of this fact will have to be considered by those who believe in the mechanical theory of atherosclerosis and especially by those who may find in experimental conditions material for their clinicopathological speculations. Whatever it may be, these experiments, positive as far as the production of atherosclerosis of the aorta is concerned, are far from satisfying the criterion I have given for a pathologic high blood pressure in rabbits (4).

Analysis of Van Leersum's Work (*2*).—Van Leersum does not mention the number of animals examined, or the length of time during which they were examined. He contents himself with saying that with his method of measuring the blood pressure (an excellent method, I believe) he has determined "regularly and for a very long time" the blood pressure oscillations of normal rabbits and of rabbits subjected to liver feeding. But there is no explicit mention of the actual oscillations found.

He says that all values were recorded, but the first ones usually were not taken into account; that only a series of 5 values which did not differ very much from one another were considered admissible (page 416), but this procedure is arbitrary and Van Leersum does not justify it. The criterion which guided him is as follows: "Die im Anfang erhaltenen Werthe jedoch sind wegen der Unruhe des Thieres in der Regel weniger gelichmässig: der Blutdruck ist dann oft bedeutend erhöht oder ermässigt. Während des Messens kommen die Thiere aber allmählich zur Ruhe und wird der Blutdruck gleichmässiger." For instance, on page 417, where a sample of a protocol is given, 11 figures (9 a.m.) are discarded, from 182 to 205 cm. water, and 5 retained, from 195 to 199 (average 196); at 2 p.m., 5 figures are discarded, from 163 to 170, and the average of the retained figures is 159. It results from all this, that Van Leersum's curves do not repre-

sent the fluctuations of blood pressure of his animals, but are constructed from sets arbitrarily selected among a wide range of values.

He reports the blood pressure of eight animals to which he gave powdered liver and of four more to which he gave sodium taurocholate or sodium glycocholate.

The summary of his results is as follows: No. 87, "average" blood pressure during normal feeding (1 week), 181.6 cm. water; during liver feeding (another week), 194.5 cm. water. No. 57 was given liver every other week. The values (means) as they appear in the graph (Curve 1) are as follows, normal feeding weeks being placed in parentheses: (163) 158 (152) 164 (156) 156 (144) 164 (162) 173 (159) 162 (156) 158. With the exception of 173, which occurs during the liver feeding, all the others are equal to or less than the first figure 163, which happens during a normal period; for differences of 1 cm. of water (less than 1 mm. of Hg) lie within the experimental error. The instance in which the average blood pressure was 173 is less than the average blood pressure of No. 87 during the control period. Rabbit F, early in October, showed an average of 164 cm. (1 week), and in the 1st week of November, still under normal feeding, 180 cm. In the 1st week of liver feeding the average blood pressure was 173, and in the 4th week 181 cm. Rabbit G, early in October, had an average blood pressure of 162.5 cm.; in the 1st week of November, 169 cm. At this time the liver feeding was started. In the 3rd week of this feeding the pressure averaged 171 cm., and in the 6th week 189 cm. water. Van Leersum adds: "Eine Steigung also von gut 11 pCt." But Rabbit F, just mentioned, jumped from 164 to 180 cm., an increase of 10 per cent, without the help of liver feeding. The other four animals may be considered jointly (A, C, D, 23). The averages during the normal feeding period varied between 141 (D) and 172 (C), the observation lasting 1 week for C, 2 for A and D, and irregularly in July, Sept., and Dec., 1911, for No. 23. The maximum weekly average recorded, *during a diet period*, appears as follows: 206 (A), 219 (C), 216 (D), 198 (23) and *after a liver feeding*, 221 (D), in the week following the cessation of the abnormal nourishment.

Van Leersum discusses these findings in the next 3 pages and then comes to the question of what part of the liver is responsible for this effect on the blood pressure. He tries the bile salts (sodium taurocholate and sodium glycocholate) and says: "ihre Wirkung auf den Circulationsapparat ist eine lähmende und sie vermindern den Blutdruck, wie Versuche an vier Kaninchen mich gelehrt haben, in erheblichem Maasse." The protocols are brief but very instructive. I copy from them the pertinent figures. Blood pressure during control period, average, Rabbit Q, 200 cm. water; Rabbit Z, 221; Rabbit X, 208; Rabbit Y, 220. Blood pressure after about 1 month of bile salt (mixed with the food, carrot), average, Rabbit Q, 168 cm. water; Rabbit Z, 190; Rabbit X, 161; Rabbit Y, 182. In other words, figures like 161, which in the main part of the paper are considered normal, are now interpreted as due to the injurious effect of the bile salts, and figures between 200 and 221 which were interpreted before (Rabbits A, C, D, 28) as due to the effect of liver feeding are now considered

normal. If Van Leersum had examined more normal animals and for a longer time, before any experiment was undertaken, he would have interpreted his results differently. I venture this statement because my figures for normal animals include *all* of Van Leersum's figures. 122 cm. water (Curve 6, Nov. 2) and 239 cm. water (Curve 2, Jan. 10) are the lowest and highest figures, respectively, recorded in the papers under discussion, that is, about 90 mm. and 176 mm. Hg respectively. In Fig. 2, Rabbit 489, Graph IV of a previous paper (3), there may be seen a few instances of pressure about 180 (averages, since Van Leersum's figures are averages also) and in Figs. 3 and 4, Rabbit 483, Graphs I, II, and III, there are several below 90.

The analysis may be summarized thus: (1) Van Leersum's range for the normal blood pressure in the rabbit, as recorded by his method, is confirmed; (2) Van Leersum's conclusion concerning the influence of a liver diet on the blood pressure of the rabbit is not substantiated by his data, since the fluctuations of blood pressure he obtained do not surpass his own recorded figures for normal animals.

Note on the Results Obtained by Other Methods.—The work of Fahr, Schmidtmann and Schönheimer, together with an account of the methods used by these authors to measure the blood pressure, has been summarized by Shapiro and Seecof (12), who in their own experiments used Anderson's method. I have answered their criticism of Van Leersum's method elsewhere (4). A few remarks, however, may not be out of place here, particularly since a few reports have appeared subsequently to Shapiro and Seecof's.

1. Van Eweyk and Schmidtmann (11) state that the blood pressure of a healthy rabbit, as recorded by their method, lies between 90 and 100 mm. of Hg. They do not say how many animals they have examined or for how long a period. By comparing their method with the values obtained with a Cowl-Gad's manometer, they find an agreement between their figures and the minimal pressure of the "blutige" measurement. But they add: "Zunächst lassen wir es dahingestellt sein, ob diese Übereinstimmung gesetzmäzig ist oder nicht." In Schmidtmann's first report (13) (six rabbits) the blood pressure was taken *once weekly*. The lowest figure is 88 (Protocol IV) before the experiment, the highest 132 (Protocol VI) in the 2nd and 3rd weeks of liver feeding. In her second report (14), she speaks of feeding experiments (diets rich in cholesterol) on 67 rabbits: in some the blood pressure effect was negative, in the large majority, however, she states that the pressure rose to 120–140 mm. Hg for weeks and even months. Then the blood pressure fell to normal, whereas the blood cholesterol remained high. She explains this fall by assuming an injurious effect of cholesterol on the heart muscle and the vascular system. It is difficult to interpret these data because it is not at all clear what is being measured with that method. The authors think that their figures agree with the minimal pressure of the aorta, but have left "undecided" (their words) how legitimate this agreement is. On the other hand, *one* measurement *once* a week gives entirely too little information on the blood pressure of the rabbit, to draw positive conclusions therefrom.

Schönheimer (8) says that he has also found an increase in blood pressure (Schmidtmann's method) under the influence of cholesterol feeding, but he gives no data, except that the largest increase was from 80 to 112 mm. Hg.

Anitschkow (15) says he has confirmed Schmidtmann's results, but gives no details, not even mentioning the method used.

Deicke (16) reports his findings on 88 rabbits fed on cholesterol or liver. He used Schmidtmann's method, but there is no statement as to how often or how many times the blood pressure was measured. Judging from his graphs, the blood pressure was taken *once a week*, sometimes more than a week apart, sometimes less. The normal curve reproduced shows fluctuations between 96 and 110 mm. The highest values represented in his graphs are, in mm. Hg, 131 (Curve 5, liver feeding), 132 (Curve 6, after intravenous injection of cholesterol solution), 133 (Curve 3, enteral cholesterol), 142 (Curve 2, enteral cholesterol).

Thölldte (17) states that the blood pressure of the rabbit, taken *twice daily* for months (Schmidtmann's method, cholesterol feeding) shows no increase "beyond the physiological fluctuations." Unfortunately he gives no details or figures, so that it is not known what is meant by physiological fluctuations.

2. Anderson's method (18) consists in the recording of the pressure necessary to obliterate the pulsation of one of the ear arteries of the rabbit. The greatest error comes, according to Anderson, from the changes in the caliber of the vessel, but he says that they can be largely controlled by keeping the ears warm. The blood pressure, in his normal animals, ranges between 75 and 90 mm. Hg. That the conditions laid down for the measurements must be closely adhered to is obvious from the recent report of Behrens (19), who devised a method essentially the same as Anderson's, and says that the values obtained in this fashion are very constant and lie about 40 mm. Hg. He does not mention Anderson's work, but quotes the work of Kuraya (20), who, independently of him, designed the same method of blood pressure measurements and obtained the same values (mean blood pressure in healthy animals 35–50 mm. Hg). Shapiro and Seecof (12) used Anderson's method and concluded that "the systolic blood pressure of the central artery of the rabbit's ear averages between 75 and 90 mm. Hg as reported by Anderson." In their table the figures for the controls are not essentially different, ranging between 77 and 105 mm. Hg. They fed lanolin to rabbits which had been subjected to various surgical operations (splenectomy, thyroidectomy, double adrenalectomy and combinations of double adrenalectomy with splenectomy or thyroidectomy). The number of blood pressure readings was very small: from 2 to 6 during the whole course of the experiments. The conclusion reached was that there is no significant hypertension during the developmental stage of experimental lanolin atherosclerosis in rabbits.

SUMMARY.

Egg yolk was fed to five rabbits provided with a good carotid loop (Van Leersum's method). The blood pressure was measured daily

before and during the egg feeding until the animal's death. The duration of the experiment varied from 81 to 391 days. The number of eggs consumed varied from 40 to 531. Two animals received, in addition to the egg yolk, lead carbonate by mouth.

Autopsy findings, blood pressure readings and pulse rate are discussed. Two blood pressure graphs are reproduced. The paper contains a brief analysis of Van Leersum's work on liver feeding and a review of the results obtained by other methods of measuring the blood pressure in the rabbit.

CONCLUSIONS.

1. Van Leersum's range for the normal blood pressure in the rabbit, as recorded by his method, is confirmed.

2. Van Leersum's conclusion concerning the influence of a liver diet on the blood pressure of the rabbit is not substantiated by his data, since the fluctuations of blood pressure he obtained do not surpass his own recorded figures for normal animals.

3. Fluctuations of systolic blood pressure beyond the "normal" range are not *necessary* for the production of experimental atherosclerosis of the aorta in rabbits. Inversely, egg yolk feeding experiments in rabbits in which atherosclerosis of varying degree, even extreme, is obtained, are not accompanied by an elevation of blood pressure *outside* the "normal" range.

4. The fluctuations of blood pressure observed during experimental atherosclerosis do not simulate the condition of essential hypertension in man.

I am indebted to Dr. G. N. Stewart for guidance and inspiration in the course of this work.

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STUDIES ON THE BIOLOGY OF STREPTOCOCCUS.

VII. Allergic Reactions with Strains from Erysipelas.

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Dochez and Sherman (1) have recently described sensitization reactions which could be neutralized by immune serum. These reactions were obtained in guinea pigs and rabbits which had been previously inoculated with living cultures of *Streptococcus scarlatinæ* or with filtrates of this streptococcus. About 10 days after the inoculation, intracutaneous injections of filtrate of cultures of scarlatinal streptococcus caused local edema and erythema. The reaction was not obtained with filtrate which had been heated or with mixtures of filtrate and scarlatinal immune serum.

Some strains of hemolytic streptococcus recovered from erysipelas produce toxic substances similar in certain respects to the substances produced by strains from scarlet fever. Cutaneous reactions may be obtained in man with the filtrates of the strains from erysipelas. The reactions obtained with a majority of the filtrates from these strains are not neutralized by scarlatinal sera but may be neutralized with sera prepared with erysipelas strains. Having this similarity in mind, we have studied the cutaneous reactions in rabbits during immunization with filtrates of strains from erysipelas.

Methods and Experimental Data.

Two strains of hemolytic streptococcus, EA and EM, were selected for the immunization of the rabbits employed in these experiments. One strain was obtained from facial erysipelas, and the second from a blood culture on a patient with phlebitis. Filtrates from cultures of both strains caused local erythema when injected intracutaneously in children and adults. The strongest reactions were obtained in children with filtrates from 48 hour cultures in tryptic digest broth.

Reactions were not obtained with filtrate which had been heated 2 hours in the Arnold sterilizer. Scarlatinal antitoxin added to the filtrates before injection did not modify the size or intensity of the erythema following intracutaneous inoculation, but the serum from rabbits immunized with erysipelas filtrates completely neutralized some of the reactions occurring with a 1/250 dilution of the filtrate.

A number of normal rabbits were tested with 0.1 cc. of undiluted filtrate before sensitization. About 20 per cent showed an indefinite erythema at the site of the intracutaneous inoculation, but none of the reactions observed at this time was as intense as the mildest reactions occurring later during immunization. Following these preliminary tests, six of the rabbits were inoculated intracutaneously with 1.0 cc. of undiluted filtrate at intervals of about 12 days. On the day preceding each inoculation skin tests were done with 0.1 cc. of the undiluted filtrates of cultures of the two strains EA and EM. Control tests were done with filtrate heated 2 hours at 98°C.

After the third inoculation edematous reactions were observed in four of the six rabbits at the site of the cutaneous injection of the filtrate. Edema and erythema occurred about 24 hours after 0.1 cc. of the undiluted filtrate was injected into the skin. This erythema lasted approximately 48 hours. The first positive reactions were observed about the 30th day after immunization was begun and occurred only with unheated filtrate. At this time the heated filtrates did not cause reactions. After the fourth inoculation erythema and some edema occurred with the heated as well as the unheated filtrate. After the fifth inoculation all the reactions were less intense than those previously observed and after the 57th day strongly positive reactions were no longer obtained. Two rabbits showed no sensitization during this period of observation.

Intracutaneous inoculations of 1.0 cc. of filtrate were continued until seven or eight injections had been given. During the interval between the time that the animals last showed cutaneous sensitization (approximately the 57th day) and the final intracutaneous inoculation two of the rabbits showed a transient recrudescence of activity. During this recrudescence the reactions obtained were exceedingly mild. After the seventh or eighth intracutaneous inoculations which were given between the 80th and the 100th days, filtrates were injected intravenously. 5 cc. and later 10 cc. were given at intervals of 2 weeks over a period of 3 months.

On the 187th day after the first intracutaneous inoculations, when the animals had been immunized for 3 months intracutaneously and 3 months intravenously, cutaneous reactions were again done. At this time all the rabbits, including the two which had previously shown no cutaneous hypersusceptibility reacted strongly with the unheated and heated erysipelas filtrates and with a filtrate of a scarlatinal streptococcus. Tests were also done at this time with filtrates of a strain of hemolytic streptococcus from a normal throat and with broth. Only one of five rabbits reacted with the throat strain. Two of the five gave questionable reactions with broth. None of the reactions was neutralized by the addition of scarlatinal or erysipelas immune sera to the filtrates previous to the injection. Serum was

TABLE I.

Cutaneous Allergic Reactions with Filtrates of Strains of Hemolytic Streptococcus from Erysipelas during the Immunization of Rabbits with Filtrates of Erysipelas Strains.

Intervals	Rabbit I					Rabbit II					Rabbit VI					Rabbit X				Rabbit XIV					Rabbit XVI				
	Cutaneous reactions					Cutaneous reactions					Cutaneous reactions					Cutaneous reactions				Cutaneous reactions					Cutaneous reactions				
	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated	Method of inoculation	Filtrate A unheated	Filtrate A heated	Filtrate M unheated	Filtrate M heated
days																													
1	m	−	−	−	−	m	±	±	±	±	m	+	−	+	−	m	−	−	−	−	a	±	−	±	−	a	−	−	−	−
12	m	−	−	−	−	m	±	−	±	−	m	+	−	±	−	m	−	−	−	−	a*	±	−	−	−	a*	−	−	−	−
24	m	±	−	±	−	m	±	−	−	−	m	−	−	−	−	m	−	−	−	−	a*	+	−	−	−	a*	−	−	−	−
34	m	++	+	−	−	m	+++	−	++	−	m	+++	±	++	−	m	−	−	−	−	a*	±	−	−	−	a*	−	−	−	−
46	m	+	−	−	−	m	++++	++	++++	+	m	++	+	+	+	m	−	−	−	−	a	++	+	+	−	a	−	−	−	−
57	m	±	−	−	−	m	++	+	−	−	m	+	−	+	−	m	−	−	−	−	a	±	−	±	−	a	−	−	−	−
68	m	−	−	−	−	m	−	−	±	−	m	±	−	±	−	m	−	−	−	−	a	−	−	−	−	a	−	−	−	−
78	m	−	−	−	−	m	+	−	±	±	m	+	+	+	±	m	−	−	−	−	A	−	−	−	−	A	−	−	−	−
92		−	−	−	−		−	−	−	−		±	−	−	−		−	−	−	−	A					A				
115	M	−				M	−				M	−				M	−				A*	−				A*	−			
123	M					M					M					M					A					A				
136	M					M					M					M														
153	M					M					M					M					A*					A*				
176	M					M						Died of respiratory disease				M					A*					A*				
187		++	+++				+++	++									+++	++												
210		+++	+++				+++	++									++++	++++				++++	++++				++++	++++		

Six rabbits were immunized with filtrates of two strains of hemolytic streptococcus from erysipelas. Filtrates of these strains have been designated by the letters A and M corresponding to the strain with which the filtrates were prepared. The rabbits were first inoculated intracutaneously and later intravenously at the intervals indicated in the first column on the left. Intracutaneous filtrate and intravenous filtrate are indicated by small and capital letters respectively in this table. Inoculations followed by an asterisk were given only approximately at the interval indicated in the first column. Preceding each inoculation cutaneous reactions were done with heated and unheated filtrates A and M. Each rabbit was tested with these four preparations. The reactions resulting from these intracutaneous tests are indicated by plus signs. Plus signs in heavy type have been used for edematous reactions.

obtained from each animal at this time and later tested for neutralizing substances in a second series of hypersusceptible animals. After the serum was obtained, desensitization was attempted with filtrates of scarlatinal and erysipelas strains, and with broth. 10 cc. of filtrate or broth were given intravenously 24 hours previous to intracutaneous tests. Desensitization occurred with the streptococcal filtrates but not with broth.

TABLE II.

The Neutralization of Cutaneous Allergic Reactions in Rabbits Sensitized with Filtrates of Strains of Hemolytic Streptococcus from Erysipelas.

Sensitized rabbit tested	Reaction with 0.1 cc. of Filtrate M	Filtrate M and Serum I		Filtrate M and Serum II		Filtrate M and Serum X		Filtrate M and Serum XIV		Filtrate M and Serum XVI	
		Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b	Mixture a	Mixture b
28	++++	+++	+	+++	−	++++	+	++++	−	+++	±
27	+++	++	±	−	−	++++	±	+++	−	+++	−
26	++	+	−	−	−	++	−	++	−	++	−
25	+	+	±	−	±	+	−	+	−		±
29	+	±	−	−	−	±	−	±	−	−	−
24	+	+	−	−	−	+	−	±	−	−	−

Six rabbits were sensitized with filtrates of strains of hemolytic streptococcus from erysipelas. The numbers of these rabbits are given in the first column. When the rabbits first showed cutaneous reactions with 0.1 cc. of erysipelas filtrate, neutralization of these reactions was attempted by the addition of immune erysipelas sera obtained from rabbits immunized by intracutaneous and intravenous injections of filtrate from erysipelas strains to the filtrate previous to injection. The sera of five immunized rabbits (Table I) were tested for neutralizing qualities. Each serum was added to filtrate of Strain M. Two proportions of serum and filtrate were employed, Mixture a (serum one part and filtrate four parts) and Mixture b (serum and filtrate equal parts). The rabbits showed no reaction with heated filtrate at the time the neutralization tests were done. Edematous reactions have been indicated in heavy type.

The sera obtained from five of these immunized rabbits were tested for neutralizing substances in a second series of sensitized rabbits. Twenty rabbits were given intracutaneous injections of erysipelas filtrate at weekly intervals until they were sensitive to cutaneous inoculations with 0.1 cc. of the filtrate. Only eight of the twenty rabbits became allergic. The neutralization tests were carried out as soon as allergy developed. Two mixtures of each serum and the filtrate—one part of serum and four of filtrate, and equal parts of serum and filtrate—were pre-

pared and incubated 3 hours. Each of the allergic rabbits was then tested with the two proportionate mixtures of each of the five sera and the filtrate. The amounts of the mixtures used were so graduated that 0.1 cc. of filtrate was injected. Although neutralization occurred with both proportions of sera and filtrate, it was most complete and uniform with the mixture containing equal parts. The neutralizing properties of the immune sera were not uniform, but no relationship was discerned between the strength of the serum and the degree of hypersusceptibility to filtrate shown by the rabbit from which the serum was obtained.

When these neutralization experiments were carried out, these rabbits reacted with unheated erysipelas filtrate but not with heated. 2 weeks later positive reactions were obtained with heated erysipelas filtrate and with filtrates of scarlatinal strains. At this time neutralization of the reaction by the addition of immune serum to the filtrate was questionable. These animals were desensitized with scarlatinal and erysipelas filtrates intravenously. 10 cc. of broth did not have this desensitizing effect.

The results of these experiments have been tabulated. Table I gives the intervals between the injections of filtrate, the method of inoculation, whether intracutaneous or intravenous, the filtrate employed, and the results of the cutaneous tests. Edematous reactions are indicated by heavy type. The neutralization tests with immune erysipelas sera and filtrate have been arranged in Table II.

DISCUSSION.

Allergic Phases during Immunization.—The rabbits which we have immunized apparently passed through consecutive periods of sensitization—a negative period from the first inoculation to the appearance of the first positive reactions, a positive allergic phase of approximately 4 weeks' duration, next a prolonged period of inactivity, and finally a second phase during which they showed cutaneous sensitization. The first period of negative reactions need not be discussed; it is observed previous to all hypersusceptible reactions before the development of sensitivity. In the animals studied this period varied from 2 to 4 weeks. The second, third, and fourth periods require discussion.

The second period was one of positive hypersusceptible reactions. This period is divided into an early phase of short duration, during which the reactions were neutralized by the addition of immune serum to the filtrate, and a later phase when the reactions were not neutralized. During the first part of this phase when the reactions were

neutralized reactions occurred only with unboiled erysipelas filtrate. The reactions observed later when they were not neutralized by immune serum were less specific, and could be obtained with heated erysipelas filtrate and with scarlatinal filtrate. The most intense reactions were obtained with the erysipelas filtrates.

The third period was a phase of inactivity of uncertain duration. This third phase began about the 68th day when hypersusceptible reactions were no longer obtained. The transition from the second period to this non-allergic state was gradual. The intensity of the reactions gradually diminished from about the 40th day of immunization until the 68th day when not even the slightest erythema occurred after the intracutaneous injection of filtrate. This gradual fading of hypersusceptible reactions has been observed by Zinsser and Grinnell (2) with streptococcus and by Mackenzie and Woo (3) with pneumococcus. Except for the positive reactions occurring later, this negative phase might be considered a return to the normal non-allergic state, or as Zinsser suggests, a state of immunity to the allergin due to the presence of antibodies.

The fourth phase was a positive phase in which the reactions with heated and unheated erysipelas filtrate and with scarlatinal filtrate were equally intense. This phase differed from the first positive phase in three respects; the reactions could not be neutralized, unheated and heated filtrate gave reactions of equal intensity, and all animals, some of which did not become hypersusceptible during the first allergic period were found hypersusceptible at this later time. During this period some of the animals were sensitive to horse serum.

Possible Factors Responsible for Allergic Reactions.—The simplest explanation of the various phases of allergy observed in these experiments is the assumption that the phases are the result of hypersusceptibility to different allergins. Dale and Hartley (4) have shown that when serum albumen and globulin are employed to sensitize animals, the intervals required for sensitization are not identical. This would appear to be a satisfactory explanation for the early and late periods of allergy. The somewhat different character of the reactions observed during the two periods supports this hypothesis.

We have already observed that allergic reactions occurring between the 30th and the 60th days of immunization could be neutralized with immune erysipelas serum up to about the 15th day after positive

cutaneous reactions first occurred. Except for the report of Dochez and Sherman we know of no authenticated instance where cutaneous allergic reactions with bacteria have been neutralized with immune serum. Reactions occurring in animals sensitized with the bodies of tubercle bacillus, staphylococcus, streptococcus, pneumococcus, and the Gram-negative pathogens or with extracts of these bacteria have never been satisfactorily neutralized. The filtrates of streptococci from scarlet fever and erysipelas contain toxic substances which are easily separated from the bacterial cells. In one respect these toxic substances resemble the exotoxins of the *diphtheriæ* and tetanus bacilli in that a satisfactory antitoxic serum can be developed by the immunization of animals. Von Behring observed sensitization to diphtheria toxin in 1893. Since this observation allergy to other bacterial exotoxins has been produced experimentally in animals. In view of these previous instances of allergy to exotoxins, and the neutralization of the cutaneous allergic reactions with immune serum in our experiments it seems possible that these cutaneous reactions are sensitization reactions to the toxic substances in the erysipelas filtrate. It also seems probable that the toxicity of these streptococcus products for man is due largely to a state of hypersensitiveness. The less specific reactions occurring later during this first period of sensitization could not be neutralized. These late reactions appear analogous to the sensitization occurring with the bodies and cell extracts of bacteria.

Experimental evidence at the present time indicates that bacterial antigens are of a composite nature and that two substances at least are linked together in the bacterial cell. Zinsser has found that precipitates from extracts of tubercle bacillus contain a non-protein substance designated the "residue antigen," and a second fraction containing nucleoproteins. These fractions are comparable to the S and P substances which Avery and Heidelberger (5) have obtained from the pneumococcus. The S substance is non-antigenic and incapable of inducing allergy. The P substance is antigenic. Animals immunized with this fraction become allergic, and from past experimental work it is probable that this fraction or some substance intimately associated with it is responsible for the allergic reactions ordinarily observed in animals sensitized with bacteria, or to the protein fractions of bacterial extracts.

Rather than assume that two substances were concerned in the first allergic phase, the reactions might also be explained by assuming that the allergin were a complex substance similar to the SP antigen in pneumococcus. The toxic fractions of streptococcus filtrates would be analogous to the SP combination. Allergic reactions to this toxic antigen would be neutralized by immune sera, while reactions due to the P analog from which the specific toxic S element had been separated would be non-specific and not subject to neutralization. As with pneumococcus antigens, reactions would occur with the SP combination and with the less specific P antigen during the immunization of animals.

Possible Relationship of Allergic Reaction to the Symptoms of Disease Due to Hemolytic Streptococcus Producing Toxic Substances.—Bristol (6) has recently drawn attention to the hypothesis that the rash and clinical symptoms of scarlet fever are allergic reactions to the products of hemolytic streptococcus. He has carefully reviewed the literature and summarized the evidence presented previously by other authors having a similar opinion. Our sensitization experiments with streptococcal filtrates afford a possible explanation of the rash of scarlatina on this allergic basis.

Infants apparently do not react to intracutaneous injections of scarlatinal filtrate. In adults negative cutaneous tests have been explained by the presence of circulating antitoxin, but Cooke (7) has found that negative reactions occur in infants whose sera have no neutralizing properties. In view of the allergic reactions which Dochez and Sherman have observed in animals hypersusceptible to *Streptococcus scarlatinæ* and the reactions which we have obtained with strains from erysipelas, we suggest that the positive cutaneous reactions with filtrates of streptococcus containing toxic fractions as well as the rash in scarlet fever are the result of previous sensitization. The development of a natural immunity to toxic fractions of scarlatinal filtrates might be explained satisfactorily by this hypothesis. Infants previous to sensitization would react negatively to the toxic substance in the absence of circulating antibody. After sensitization, positive cutaneous reactions would occur accompanied by a susceptibility to rash in event of infection with *Streptococcus scarlatinæ*. With the development of sufficient circulating antibody the

positive reactions would become negative and the tendency to develop the rash would disappear. In this immune state scarlatina *sine* exanthem might occur.

CONCLUSIONS.

Rabbits immunized with filtrates of cultures of hemolytic streptococcus from erysipelas show cutaneous allergy. Two periods of allergy have been observed, an early and a late phase. The earliest reactions occurring in the first period of allergy can be neutralized with erysipelas immune sera.

The rash of scarlet fever and the Dick reaction are apparently allergic reactions to products of *Streptococcus scarlatinæ*.

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STUDIES ON STREPTOCOCCUS BACTERIOPHAGE.

I. A Powerful Lytic Principle against Hemolytic Streptococci of Erysipelas Origin.

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INTRODUCTION.

The influence of the bacteriophage upon streptococci was studied by several authors. Piorkowski (1) was the first to report a lytic principle against streptococci. He mixed 2 cc. of blood from a patient suffering from subacute bacterial endocarditis with glucose broth and filtered this mixture after 4 days incubation. The filtrate showed lysis of the streptococcus obtained from the blood of the same patient. The lytic principle was, according to Piorkowski, transmissible. The qualitative and quantitative potency of this principle was not studied, or were observations made as to whether the principle had any effect upon other pathogenic streptococci.

McKinley (2) claimed that he was able to obtain a lytic principle against a large group of Gram-negative and Gram-positive cocci, including streptococci. To demonstrate such a principle he filtered a given culture containing anti-b. Shiga phage, reinoculated the filtrate, incubated, and filtered again. This was repeated three or four times in succession. When 5 cc. of such a filtrate, diluted 1:10 with broth, was inoculated with the organism from which it was obtained, it failed to grow. This author did not do any experiments on the properties of this principle; namely, on the qualitative and quantitative potency, formation of resistants under its influence, and regeneration of this principle by the resistants. It would be impossible, therefore, to accept these inhibitory factors as evidence of bacteriophage.

Dutton (3) studied the appearance of colonies of various strains of

streptococcus. A large number of strains contained irregular and "moth eaten" colonies and, therefore, suggested symbiotic existence of bacteriophage. Only one strain had proven itself susceptible to a lytic principle obtained from another strain which failed to grow on transplant. The evidence brought forward suggested the existence of streptococcus bacteriophage of a low order which in the majority of instances is not able to produce the classical picture of the bacteriophage phenomenon.

Further work was reported by Hadley and Dabney (4). These authors were able to obtain a potent bacteriophage for one strain of *Streptococcus fæcalis*.

Clark and Clark (5, 6) obtained from activated sludge a bacteriophage which was highly potent against two strains of hemolytic streptococci isolated from rabbit and guinea pig lung infections. They failed to adapt this principle to five strains of human pathogenic streptococci.

It is evident that great difficulties accompany attempts to produce an active lytic principle against streptococci. The isolated positive results with a few strains, only two of which were of human origin (Piorkowski (1) and Dutton (3)), leaves the field open for further investigation. The question therefore arises as to whether streptococci as a genus are susceptible to the bacteriophage phenomenon or whether the few strains of streptococci in which this phenomenon was observed carried a factor, not common to other streptococci as a genus, which enabled them to be affected by the phage. With these considerations in mind the author attempted to obtain an active principle against pathogenic human streptococci. The following plan was adopted for this work:

1. To study the effect of Clark and Clark's "sludge" bacteriophage upon the susceptible strain of rabbit streptococcus in order to determine the properties of this principle, its specificity and relation to the classical bacteriophage phenomenon.

2. To make attempts to obtain a powerful bacteriophage for pathogenic streptococci by means of the Clark and Clark phage as well as by other lytic principles.

3. Should these attempts succeed, to study the properties and specificity of such a bacteriophage.

EXPERIMENTAL.

Lytic Principles Tested against Pathogenic Streptococci.

The following lytic principles tested on various occasions against streptococci will be mentioned in the course of this paper:

B.H.—A lytic principle kindly sent to me by Dr. A. Gratia, isolated by him from vaccinia pulp. This principle was potent against numerous strains of *Staphylococcus aureus* and *albus*.

Anti-B. coli Phages.—A large series of extremely potent bacteriophages was obtained by the author from stools of various animals. They will be mentioned under the name of the animal from which they were obtained.

L_{12} and C/2.—L_{12} represents anti-b. Shiga bacteriophage kindly sent to me by Dr. J. Bronfenbrenner. C/2 is the same phage but adapted to *B. coli*.

"Sludge" phage.—This principle was isolated by Drs. Clark and Clark from activated sludge and kindly sent to me a year ago. It proved itself extremely active against a *Streptococcus hæmolyticus* isolated by the same authors from a rabbit. The susceptible strain will be referred to as "Rb" streptococcus.

Strains of Streptococci Employed in This Work.

Numerous strains of hemolytic and green-producing streptococci employed in this work were isolated from various sources in this laboratory. Others were kindly sent to me by Dr. A. W. Williams, Dr. Konrad Birkhaugh, and by the American Type Culture Collection. The nature of these strains will be described as the occasion arises.

I.

Observations on general properties of "sludge" phage corroborate Clark and Clark's report (6). The following requirements which are necessary to identify this principle with the classical bacteriophage can be fully answered:

1. Lysis can be demonstrated on solid media (in the form of so called "eaten up" areas) and in fluid media. The principle diluted to 1×10^{-8} cc. is still able to produce complete inhibition of growth. After lysis occurs such a culture shows again the full concentration of undiluted lytic principle.

2. Lysed culture eventually gives rise to growth. Such a growth is able to regenerate bacteriophage in turn when transplanted into a fluid medium.

3. Formation of resistants is accomplished by prolonged cultiva-

tion of susceptible streptococci with at first highly diluted and then undiluted lytic principle.*

4. Immunization of rabbits with lysed cultures brings about formation of antibacteriophage serum.

5. In addition it should be mentioned here that this principle is less stable than *coli* dysentery phages. When "sludge" phage is heated to 58° for ½ hour its lytic exponent (E_L) is reduced from −8 to −3. Control heating of dysentery phage to the same $t°$ does not affect it whatsoever. The lytic potency is also liable to become greatly reduced on standing in the refrigerator for 2 to 3 weeks.

6. The effect of "sludge" phage on other streptococci was studied in fluid media and observations were made 24 hours and 2 to 3 days later. In the course of 1 year 102 strains of pathogenic streptococci of human origin were tested, as follows: 12 strains of the group of scarlet fever hemolytic streptococci; 18 strains of *Streptococcus erysipelatis;* 3 strains isolated by Tunnicliff from cases of measles; 1 strain of septic sore throat, 1 strain from Rosenow's ulcerative colitis case; 1 strain from Rosenow's case of acute anterior poliomyelitis; 12 strains of green-producing streptococci from various human infections; 16 strains of anhemolytic streptococci of human origin, and 38 strains of hemolytic *pyogenes* strains. In not a single instance did "sludge" phage show the slightest suggestion of lysis. The specific lytic effect of this bacteriophage only upon the two strains of streptococcus mentioned above is very striking. It was of interest therefore to study the nature of this resistance as compared to the same phenomenon encountered in various strains of bacteria generally susceptible to the phage.

From the studies on the bacteriophage phenomenon it is evident that two types of resistance to phage can be differentiated.

1. *Apparent Resistance.*—Among the genera susceptible to phage, occasional strains can be found which do not undergo any lysis when brought into contact with a given principle. However, these strains are able to regenerate bacteriophage and carry it from generation to generation without being attacked themselves.

* The first three points were published by Clark and Clark when this work was almost completed.

2. *True Resistance.*—Certain genera of bacteria for which no bacteriophage was as yet found; or occasional strains of susceptible bacteria; or individual colonies of bacteriophage carrying strains show complete indifference to this principle. They are not attacked by it and are not able to regenerate it. In order to determine to which of these categories the resistance of streptococcus belongs, experiments were made in a quantitative manner and the fate of "sludge" phage in resistant cultures was followed up. Strains 55 (Williams—scarlet fever); E, (Birkhaugh—erysipelas); V-(*viridans*—endocarditis); H (*hæmolyticus*—meningitis) were used for these experiments. Flasks of broth containing 1×10^{-5} cc. dilution of Clark's phage ($E_L - 8$) were inoculated with these strains. In 24 hours the cultures were filtered and the filtrates tested in various dilutions against "Rb" streptococcus. All these filtrates were able to produce lysis of "Rb" streptococcus in dilutions up to 1×10^{-2} cc. only. It is evident that no regeneration of lytic principle was attained by any of the streptococci employed in these experiments. The conclusion was, therefore, that streptococci showed true resistance to lytic principle. In view of the fact that reproduction of the bacteriophage phenomenon is impossible without the ability of regeneration on the part of bacteria, it was thought advisable to direct the work towards creating favorable conditions which would enable streptococci to regenerate phage. Should these efforts fail it would then be of interest to attempt to train streptococci to perform this function.

II.

Adaptation of Various Lytic Principles to Hemolytic Streptococci.

In this experiment the usual method of "adaptation" of phage to a resistant microorganism was used. Various anti-*coli* phages, B.H. and "sludge" phage, were titrated against three strains of scarlet fever streptococci (Williams—55, 108, 130); one strain of erysipelas (Birkhaugh—E_1), and five strains of *pyogenes* hemolytic streptococci. In 24 hours each titration was then pooled in a flask, diluted with broth, and incubated for a few days. These cultures were filtered, the filtrates reinoculated with the respective strains, some lytic principle added, and the mixtures incubated again. This process of successive filtration,

reinoculation, and incubation was repeated four times. The final filtrates were tested against the above mentioned strains of streptococci. Occasionally, a filtrate used in dilution 1 : 10 would show a suggestion of lysis. However, it was never possible to duplicate such a result and all these trials remained frankly negative. Since in the majority of instances exact record was kept of the amount of lytic principle added, it was possible to determine by means of experiments described in Part I that there was never any regeneration of these principles.

The Influence of Age of Culture upon Susceptibility to the Bacteriophage.

The original lytic principles as well as various filtrates of the preceding experiment were tested against the above mentioned scarlet fever streptococcus cultures of various ages from 6 hours to 1 month old. Only in one case, "pig" anti-*B. coli* phage showed complete lysis with 1 month old culture of Strain 55 without regeneration however. It was impossible to repeat this result. All the other results were distinctly negative.

The Influence of Various Degrees of Anaerobiosis Tested at Different Hydrogen Ion Concentration upon the Susceptibility of Streptococci to Bacteriophage.

The author has previously demonstrated (7) that a quantitatively weak principle was able to produce complete lysis in culture of *B. coli* placed under partially anaerobic conditions in cases where it failed to do so under frankly aerobic conditions. Moreover (8) definite reciprocal relationship had to be observed between the hydrogen ion concentration and oxygen tension in order to enable *B. coli* to retain its susceptibility to phage. These observations were applied to the studies on streptococci with the hope that more favorable conditions would be found for obtaining lysis and regeneration of bacteriophage. For this purpose Strains 55 and 108 were cultured for three generations in broth of different pH under various oxygen tension conditions. The different cultures were then tested against several lytic principles under similar conditions. No lysis was observed in any of these cultures and no regeneration obtained.

The Influence of Temperature on Lysis of Streptococci.

Experiments with principle B. H. and staphylococcus showed the following: If B. H. was not regenerated for a period of 2 to 3 weeks it was apt to lose its potency almost completely. However, if a culture of staphylococcus containing such a principle was left at room temperature for a few days sudden complete clearing occurred. Such a lysed culture then contained a lytic principle which was able in turn to produce prompt lysis at 37°. It was decided to apply this observation to the studies upon streptococci and combine it with the method of the preceding experiment. The preceding experiment was repeated but cultures containing lytic principle were left at room temperature for over 2 weeks. No lysis appeared under these conditions and no regeneration of lytic principles was noticed.

Addition of "Activating" Factors to Cultures of Resistant Streptococci.

On the assumption that spontaneously resistant strains of streptococci may not produce regeneration on account of the absence of some activating factors, the following experiments were undertaken: These hypothetical factors were looked for in cultures of "Rb" streptococcus which always showed remarkable susceptibility. Cultures of 55,108, and 130 strains were grown in broth containing a suspension of live "Rb" streptococcus, or filtrate of live culture, or a suspension of this culture autolysed by freezing and thawing. (Autolysis was made aerobically and anaerobically.) In no instance did the strains prepared in such a manner show any change in susceptibility to "sludge" phage.

"Training" of Streptococci to Regenerate Bacteriophage.

The lack of power regeneration under various supposedly favorable conditions suggested the necessity of employing some method which would "train" the strains under question to perform this function. In the methods ordinarily employed the lytic principle was "adapted" to the microorganisms. In contrast to this method (page 501), the author attempted to "adapt" the microorganism to the lytic principle. Various strains of human pathogenic streptococci chosen for this experiment were subcultured every 24 hours in broth containing 1:10 dilution of lytic principle. After several such passages

the cultures were inoculated into flasks of broth, incubated for 24 hours, filtered, and 0.5 cc. of each of these filtrates tested against homologous normal cultures of streptococci. Table I represents the results obtained.

As is seen from this table, sixteen strains of hemolytic streptococci of erysipelas origin acquired the property of regenerating lytic principle active against normal cultures of their homologous strains. The

TABLE I.

Strain of streptococcus	Lytic principle	Number of passages through phage	Degree of lysis of normal culture *vs.* filtrate of "adapted" culture of the same strain
Birkhaugh erysipelas E_1	C/2	15	0
10 erysipelas strains (Birkhaugh) E_1–E_{10}	"Sludge"	12	4+
Erysipelas—E	C/2	36	0
Viridans subacute bacterial, endocarditis	"Sludge"	30	0
Scarlet fever 55, 108, 130, 4, 84, 42, "L", "M"	"Sludge"	20	0
Scarlet fever 55, 108, 130	"Sludge"	70	0
"Rb" streptococcus	C/2	15	0
"Rb" streptococcus	"Pig"	20	0
Pyogenes hemolytic 2 strains	"Sludge"	37	0
Scarlet fever 55, 108, 130	C/2	19	0
Scarlet fever 55, 130	"Pig"	18	0
2 strains *pyogenes* hemolytic streptococcus	C/2	31	0
Erysipelas strains Birkhaugh 243, W 248, 214, 268.	"Sludge"	12	4+
American Type Culture Collection erysipelas 766, 777	"Sludge"	12	4+
American Type Culture Collection erysipelas 768, 425	"Sludge"	12	0
769 Birkhaugh 239, 218	"Sludge"	12	0

same method failed to give any results with the same principle cultivated with other pathogenic streptococci, and another lytic principle failed to give results with the erysipelas strain. The following two explanations offered themselves to account for the failure of numerous other streptococci to acquire the property of regeneration.

It is quite possible that the strains which could be eventually trained to regenerate phage normally possessed this property in a *latent* condi-

tion before the experiment began, while the other strains which failed to acquire this property by this method—*entirely* lacked it. However, C/2 phage failed to evoke this property in a strain of erysipelas streptococcus, which was shown to undergo this change with "sludge" phage. There remained, therefore, the second possibility, namely that the phage employed must also possess a special ability to evoke such a change. The question as to whether the resistant streptococci would not eventually demonstrate the function of regeneration if only a proper phage is offered to them in the process of "training" is at present being followed up.

Streptococcus Erysipelas Bacteriophage.

The following observations were made on the erysipelas streptococcus bacteriophage:

Quantitative and qualitative estimation of potency of phage was made by titration in broth. The filtrate of a broth culture of a strain which was trained to regenerate a powerful lytic principle showed lytic exponent -4. The last tube showing lysis was filtered. The filtrate then showed $E_L - 8$. Such a lytic principle could be kept at this titer and transmitted indefinitely through contact with normal cultures of erysipelas strains. Care had to be taken to regenerate the phage frequently since the lytic principle had a tendency to partially lose its potency on standing. As is seen from these observations, the lytic principle regenerated by a "trained" strain was afterwards easily regenerated by normal cultures. Evidently, therefore, the process of obtaining phage for a resistant strain consists of the following stages:

(*a*) "Adaptation" of such a strain to the "sludge" lytic principle bringing about appearance of power of regeneration. (*b*) Change of the "sludge" lytic principle offered to the strain to a state which allows its regeneration by a normal and, therefore, unprepared culture of the same strain. To sum up the results so far obtained: (1) It is interesting to note that resistant strains of streptococcus may lack completely the ability of regeneration for one type of phage (C/2). (2) They may have a "latent" potentiality of regeneration for another type of phage ("sludge"). (3) They may have this property in an active state for the third type of phage (erysipelas phage).

The strain of erysipelas streptococcus which was "trained" to regenerate an antierysipelas streptococcus phage was studied.

1. When a subculture on solid medium was tested against streptococcus erysipelas phage it showed complete resistance as demonstrated by the absence of "eaten up" areas in those areas where a drop of phage was put on the surface of a bacterial film. Nor did any suggestion of lysis occur in fluid medium.

TABLE II.

	"Sludge" phage *vs.* Rb streptococcus	"Sludge" phage *vs.* erysipelas streptococcus	"Erysipelas" phage *vs.* Rb streptococcus.	"Erysipelas" phage *vs.* erysipelas streptococcus
Degree of lysis	4+	0	4+	4+
Lytic exponent	−8	0	−8	−8

TABLE III.

Degree of Lysis.

Dilutions of sera	−1	−2	−3	−4
Serum 87 *vs.* 0.5 cc. "sludge" phage + Rb streptococcus..	0	0	0	0
Serum 87 *vs.* 0.5 cc. erysipelas phage + erysipelas streptococcus	0	0	0	0
Serum 212 *vs.* 0.5 cc. of "sludge" phage + Rb streptococcus	0	0	4+	4+
Serum 212 *vs.* 0.5 cc. erysipelas phage + erysipelas streptococcus	0	0	0	0

4+ = complete lysis; 0 = normal growth.
Serum 87 prepared by immunization with "sludge" phage.
Serum 212 prepared by immunization with erysipelas phage.

2. The above mentioned strain was also streaked on the surface of a plain agar plate. Microscopic examination of colonies appearing in the course of 1 week did not show any striking morphological changes such as described for other microorganisms acted upon by phage.

The following colonies were observed: large smooth but slightly irregular with occasional erosions in the center or at the margin of the

colony; small smooth colonies with irregular margin; irregular fine masses of cocci without any definite colony formation. These colonies were grown in broth and tested against erysipelas bacteriophage. They all showed the same degree of resistance. The filtrates of fluid cultures contained active lytic principle. It was, therefore, impossible to obtain resistant but bacteriophage-free colonies, such as can be done with other microorganisms. A more painstaking search should, however, be made. The above observations leave no doubt that the lytic principle obtained is in no respect different from the classical bacteriophage.

TABLE IV.

Strains tested *vs.* erysipelas phage	Lytic exponent	Degree of lysis
10 strains of streptococcus erysipelas (Birkhaugh) E_1–E_{10}	−8	4+
Birkhaugh, erysipelas streptococcus 243, W 248, 214, 268	−8	4+
American Type Culture Collection 766, 767 streptococcus erysipelas	−8	4+
Williams, scarlet fever streptococcus 55, 130, 108, 4, 84, 42	0	0
Rb *Streptococcus hæmolyticus*	−8	4+
Mt. Sinai, scarlet fever streptococcus "M", "L"	0	0
Streptococcus erysipelas, Birkhaugh 239, 218	0	0
Mt. Sinai, hemolytic pyogenic streptococci, 36 strains	0	0
American Type Culture Collection streptococcus erysipelas, 768, 425, 769	0	0
Mt. Sinai, green-producing streptococci, various conditions, 8 strains	0	0
Mt. Sinai, anhemolytic streptococci, 5 strains	0	0
American Type Culture Collection anhemolytic streptococci 421, 422, 423, 349, 345 hemolytic, sore throat 543	0	0

In order to determine the relations of "sludge" phage to the erysipelas lytic principle, antiphage sera were prepared with both phages. Care was taken to "purify" the erysipelas phage by several transmissions through normal cultures of erysipelas strains. Tables II and III represent the results obtained.

As is to be noted, both phages are able to lyse "Rb" streptococcus, while only erysipelas phage is able to dissolve the erysipelas strain. It can be assumed, therefore, that both phages contain a common component and that the erysipelas phage has also a specific portion which makes possible lysis of erysipelas streptococcus. Since the "sludge" phage serum is also able to neutralize erysipelas phage it is apparent

that the presence of the common component in addition to the specific portion is essential for the action of the erysipelas phage. A difference can be detected in the effect of antierysipelas phage serum upon both phages. This serum neutralizes the "sludge" phage in dilution 1:100 and the erysipelas phage in dilution 1:10,000. No explanation is at present offered for this result.

Studies on the specificity of the erysipelas streptococcus bacteriophage are recorded in Table IV.

Thus this bacteriophage is able to attack about 76 per cent (16 out of 21) of all the tested hemolytic streptococci of erysipelas origin besides the "Rb" streptococcus. The specificity of this phage is of extreme interest. As is seen, no other streptococci out of 64 strains tested is attacked to the slightest degree. It would be of interest to study the relation of the specific agglutinogen of this group to the susceptibility to this phage.

SUMMARY AND CONCLUSIONS.

1. The "sludge" phage obtained by Clark and Clark answers all requirements for pronouncing it identical with the classical bacteriophage.

2. The "sludge" phage failed to produce lysis in any of the 102 human pathogenic streptococci tested.

3. Numerous attempts to induce regeneration of various lytic principles by human streptococci resulted in failure.

4. It was possible, however, to "train" erysipelas streptococci to regenerate a lytic principle active against 76 per cent of strains of this group.

5. The erysipelas phage showed remarkable specificity.

I wish to express my thanks to Miss G. Ethelbert McKennon for her assistance.

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THE RHYTHMIC RANGE OF THE WHITE BLOOD CELLS IN HUMAN, PATHOLOGICAL LEUCOPENIC AND LEUCOCYTIC STATES, WITH A STUDY OF THIRTY-TWO HUMAN BONE MARROWS.

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In an attempted interpretation of the occasional unexplained inconsistencies in clinical blood counting it has been suggested (1) that there is normally a more or less rhythmic variation in the numbers of white blood cells from moment to moment in each individual. In general the total number of white blood cells has been found to vary, within physiological limits and without abnormal external or internal stimuli, 100 per cent during the course of a day in a single, apparently normal, adult individual. In other words, rather than a relatively fixed individual "norm" or concentration of circulating cells, our concept of the normal for each person now is a wide zonal range, with a fluctuation for example of from 4000 to 8000 or from 6000 to 12,000 white blood cells, any chance single count being the resultant of at least three variables, the person, the physiological conditions of the moment, and the time of day of the observation. A. F. Bernard Shaw (2) has recently confirmed this concept and has carried his observations further by describing diurnal tides of the leucocytes in man, finding consistently two low points during the 24 hours, one between 9 and 11 in the morning, the other between midnight and 2 a.m., entirely independent of starvation, food, rest, exercise, or sleep. Bushnell and Bangs (3) in surveying the large individual variations in both white and red cells occurring in normal rabbits, conclude from their mean of 10,675 that $\pm$4800.75 must be taken as the limits of normal for determinations of the white cells, and from a mean of 5,989,500, $\pm$1,682,100 for the range of normal for the red cells. In one normal

animal daily and hourly counts gave as extreme limits for the white cells 10,200 and 13,600, for the red cells 4,322,000 and 6,976,000. For a review of the literature and an analysis of the normal rhythmic variations of the white cells the reader is referred to the three articles above mentioned.

The question which arises immediately in the mind of the clinician, habituated to depend more or less upon the leucocyte count in disease, is as to the dependence to be placed in single counts in the light of the demonstrated 100 per cent, normal individual fluctuation. To the end that we might possibly determine something concerning the mechanism of, and range for, the white blood cells in disease the series of studies here presented were undertaken. We have been guided in our experimental observations by an attempt to answer the following questions: Are the observed variations in total numbers of white blood cells in the same individual from time to time fancied or real? How large a factor is the error inherent in the technic of counting? Can rapid changes in blood volume account for the variable counts? How often and how regularly do the changes come? In short, is there an analyzable inherent rhythm to the fluctuation in the total white blood cells in disease, and do the different types of white cells maintain independent rhythms? If so, do they parallel the established normal variants, *i.e.* is a leucopenia simply the manifestation of a depression, a leucocytosis the result of an accentuation, of the normal mechanism? Is it important to do more than one white count for an accurate working knowledge of the available cells at any one period in the course of a disease, and, if so, how many and at what intervals should the repeated observations be made? Can we arrive at any idea of the rate and manner of delivery of cells from the bone marrow by an analysis of consecutive observations on the peripheral blood at 15 minute intervals? In summary, are the apparent fluctuations in total numbers of white cells actual changes in the absolute numbers of potentially available circulating units, or do such changes represent technical, observational inaccuracies plus a vasomotor, redistribution phenomenon with percentage importance only? Entirely irrespective, however, of the desire to understand and to explain the mechanism in these observations, is the fact that controlled consecutive counts do register large variations which must

be taken into consideration when the leucocyte count may be an important factor in diagnosis or prognosis and treatment in disease.

We have studied a wide series of clinical conditions in which both leucopenia and leucocytosis of varying degree have existed. The twenty selected cases presented in this report are but representative of similar findings in a much larger survey of cases, but in which the data are less complete. As a routine each patient has been followed for a period of 3 hours, at intervals of 15 minutes, certain cases having been observed on successive occasions for similar unit periods. This permits of the construction of a graphic record with a more accurate interpretation of the limits and meaning of the variations. An automatic lancet was used, thus theoretically insuring consecutive samples of freely flowing blood from the same depth each time. A different finger was used for each observation, or a different portion when it was necessary to use the same finger again toward the end of a 3 hour experiment. The order of procedure was as follows: first drop discarded, supravital preparation, cover-slip spreads for Wright's stain, total white blood cell count, total red blood cell count, hemoglobin, hematocrit, serum protein. In the complete studies two of us were making the preparations, each taking the same type of sample each time, so that usually within 2 minutes all tests had been secured and the individual personal error in each particular series should have been fairly constant. In several of the cases a double check of the total white blood cell count was made by two persons independently to determine the error inherent in this technic. The same Bureau of Standards equipment was used throughout in each series of observations. The hemoglobins were read in a Duboscq colorimeter with the Newcomer standard. The hematocrit determinations were made with the Van Allen pipette (4). Refractometric readings were made for serum protein determinations. Our problem has not been concerned so directly with the establishment of exact absolute values for hemoglobin, serum protein, and hematocrit readings as with the relationship existing between consecutive *ad seriatum* determinations made under identical, standardized, closely controlled conditions and bearing to each other a definite time relationship. This might be expected to give at least the relative variations in a given determination over the period of observation. In some instances a 100 cell supravital differential count was made, with vital neutral red (5, 6); in others cover-slip preparations were made with Wright's stain and 200 cells counted by two observers from both cover-slips and the average percentages taken; in some cases both supravital and fixed film observations were made and compared. We have found that while it is essential to count larger numbers of cells and average the counts from both cover-slips to obtain accurate data from fixed films, with the supravital preparations, in which the whole drop of blood is evenly and uniformly spread out, 100 white cells counted will give in the majority of instances the same percentage incidence of cell types as a larger number of cells counted or as is given by a count of two consecutive preparations by two different individuals. In certain of the cases we have made an

Arneth differential count uniting for charting the non-lobulated and two lobed nuclei as significant of the younger mature polymorphonuclear neutrophils, the three and four lobed nuclei as fully mature, and, those having five or more lobes as indicating old cells. In the supravital preparation we have differentiated the actively motile leucocyte from the round inactive form and from the specific "non-motile" neutrophil of Sabin (5), the last being interpreted as the morphological state denoting the physiological death of the cell.

In addition to the series of twenty cases, which form the basis of the clinical observations herein analyzed, differential cell counts of the bone marrow have been

TABLE I.

Case	Age	Sex	Diagnosis	W.B.C.	Range 3 hr. period with 15 min. counts
	yrs.				
1 (T71)	35	M.	Chr. osteomyelitis	Leucopenia	1,400– 2,800
2 (T44)	40	"	Primary anemia	"	2,900– 5,300
3 (T5)	65	"	" "	Low normal	4,000– 6,500
4 (T86)	25	"	Ac. rheumatic fever	Normal	4,200– 7,000
5 (T87)	22	"	" " "	"	4,200– 8,000
6 (T89)	25	F.	" " "	"	4,400– 10,200
7 (T111)	30	"	Erythrodermia	Leucocytosis	7,400– 15,100
8 (T81)	75	M.	Chr. cystitis	"	9,500– 15,500
9 (T88)	24	F.	Ac. rheumatic fever	"	8,800– 16,500
10 (T90)	18	"	Chr. otitis media	"	10,800– 16,500
11 (T41)	40	M.	Lobar pneumonia	"	10,000– 20,000
12 (T4)	55	"	Polycythemia vera	"	10,500– 23,000
13 (T92)	40	"	Amebic dysentery	"	14,700– 24,600
14 (T78)	16	F.	Pertussis	"	17,000– 25,000
15 (T82)	15	M.	Rheumatic endocarditis	"	16,000– 32,000
16 (T109)	30	"	Lung abscess	"	23,000– 34,600
17 (T110)	40	"	Septicemia (strep.)	"	23,900– 34,700
18 (T63)	30	"	Lobar pneumonia	"	50,000– 75,000
19 (T70)	60	"	Chr. lymph. leucemia cutis	"	35,000– 52,000
20 (T112)	45	"	" myeloid leucemia	"	208,000–284,000

made from thirty-two selected cases coming to autopsy in the Pathological Department of the Boston City Hospital. This has given additional independent evidence in confirmation of the probable part played by the bone marrow in white blood cell fluctuations, more spectacularly in the leucocytoses. These latter preparations were made originally (L.G.Z.) with an entirely different end in view and only on subsequent analysis was it seen how strikingly an interpretation of these data corroborates the rhythmic concept developed from a study of the peripheral white cell fluctuations in health and disease. In the postmortems performed within 5 hours after death, supravital differential studies were made to

check with the fixed films (7). However, in the majority of cases the counts were taken from fixed preparations. In all instances, except as specifically indicated, the femoral bone marrow was used. Occasionally it was convenient to examine

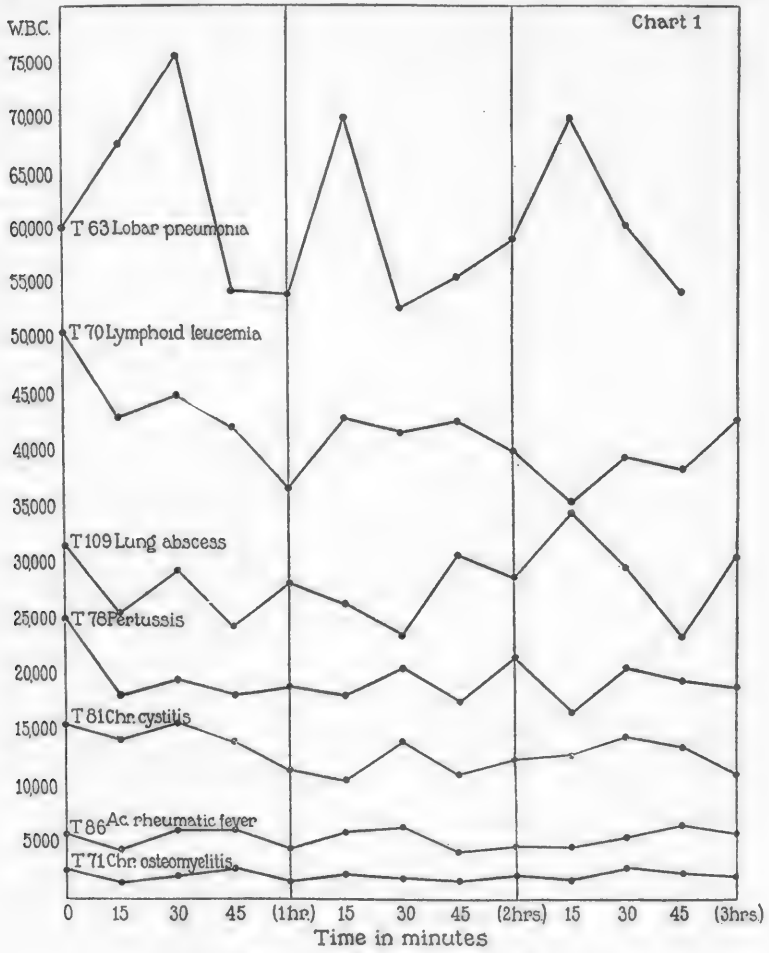


CHART 1. Fluctuations in total white cell counts.

vertebral marrow. In the empyema cases, the marrow from ribs removed at operation formed the basis for the counts. All fixed preparations were made as follows: A small piece of bone marrow was emulsified in 2½ per cent sodium citrate, and centrifuged at high speed (2600 R.P.M.) for 10 minutes. The supernatant

fluid with the fat was pipetted off and the cells were rewashed three times with sodium citrate, the suspension being centrifugalized each time. The washed cells were then suspended in a few drops of autogenous serum, from which coverslip preparations were made. After drying in the air, they were stained with Wright's stain and mounted in balsam. No fewer than 1000 cells were counted in any case and as many as 2000 cells were counted in some instances, by two observers, and the average percentage recorded.

In Table I will be found the clinical cases on which at least one 3 hour period of observation was made. The patients' age, sex, diagnosis, and range of total white blood cells during the 3 hour period are given. In Chart 1 are to be seen graphic representations of the white blood cells in selected cases showing the variations in the total white cells at different levels—from a leucopenia of from 1400 to 2800 cells to a leucocytosis of from 50,000 to 75,000.

Controls in the Establishment of the White Cell Variations.

In the attempt to establish in so far as might be possible the nature of the apparent fluctuations in the total white cell count, coincident and simultaneous observations of the total red blood cells, hemoglobin, hematocrit, and serum protein were made, all or in part, in five cases, as recorded in Charts 3A, 8A, 10A, 11A, and 12A. The curves of the red cells, hemoglobin, and hematocrit tend to follow each the other but with no relationship to the total white cell curve. The serum protein produces the most constant finding, the very slight changes noted in Chart 12A bearing no relationship to the fluctuations in the other curves, and in Chart 8A there was a constant reading throughout the whole period except for one slight rise. It has been generally accepted that the serum protein (8), under normal basic, fasting conditions is one of the most constant constituents of the blood, relatively, and by pediatricians, in particular, has been used extensively as an index of dehydration. This fact, together with the ease of refractometrically determining its per cent on small samples of blood caused us to decide to make these consecutive determinations.

Table II summarizes the range of total red blood cells and hemoglobin observed in selected cases in this series. Roughly, the hemoglobins run in percentage variation about half that of the cells, *i.e.*, 1 per cent variation in hemoglobin is the equivalent of a 2 per cent

variation in red cells. The tendency toward a relatively large variation between the minimum and the maximum count when consecutive observations on the same individual are compared, whether the time interval be in minutes or days, has been so constant in the experience of the last several years that, for the red cells too, we believe there is a normal, individual zonal fluctuation rather than an exact point in total count which it is necessary to consider. Thus when a single count, particularly in the female patient, reveals the total red blood cells near 4,000,000 it is necessary to know whether that figure represents a minimum, the average, or a maximum point in the physiological range for that individual. As is not infrequently the case,

TABLE II.

Red Blood Cells.

Range during 3 Hour Period with Counts at 15 Minute Intervals.

Case No.	Minimum count	Maximum count	Hgb. range	R.B.C. var.	Hgb. var.
			per cent	*per cent*	*per cent*
3 (T5)	3,616,000	4,808,000	74– 85	24	13
10 (T90)	4,112,000	5,232,000	90– 96	21	6
12 (T4)	5,760,000	6,470,000	125–130	10.9	4
13 (T92)	3,696,000	4,624,000	64– 72	20	11
14 (T78)	4,880,000	5,632,000	83– 89	13	7
Normal individual (R44)	5,?30,000	6,180,000	79– 88	15	10

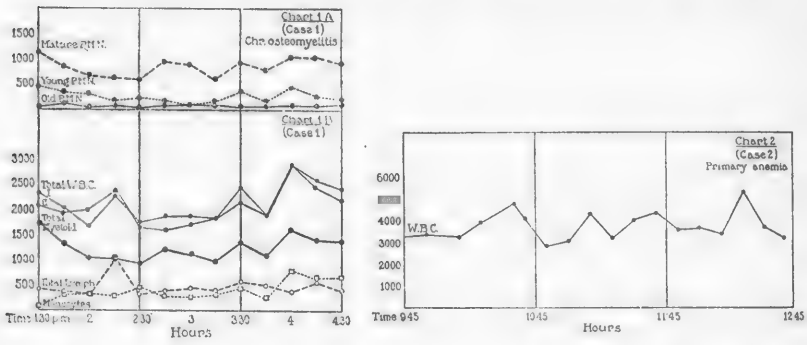
it may well be only the lower limit of a normal swing in the count for that patient if subsequent counts register 4,500,000 to 5,000,000. Only a consistent trend upward or downward in both hemoglobin and total cells as determined in repeated observations on the same individual over a period of days may provide a reliable basis for an estimate of the state of equilibrium, or the lack of it, in the supply of red cells from the hemopoietic organs. The lack of correlation between the curves of the serum protein, the white and the red cells in the present series of cases minimizes the factors of blood volume and circulatory disturbance as solely responsible for the observed variations. Rather the now well recognized reservoir for red cells in the spleen (9) and the known periodic delivery of new cells from the bone marrow (as re-

vealed, for example, in "normoblastic crises") must be considered as potential factors of importance.

Leucopenia.

The lowest maintained white blood cell count which was observed during the year was that of Case 1 (Charts 1A and 1B) in the series.

The diagnosis of chronic osteomyelitis of the right tibia was made. Repeated white blood cell counts made singly on successive days during the patient's course in the Hospital consistently registered below 4000. During the 3 hour period of the consecutive 15 minute observations the lowest number of white cells counted



was 1350, the highest 2850. In this instance two of us took white blood cell counts simultaneously and counted them independently in an endeavor to check the error inherent in the technic of counting (see also Charts 7B, 17A). Because of the marked leucopenia blood was drawn to the 1 mark in the pipettes instead of the usual 0.5. The greatest single variation from the two pipettes was 700 cells; the greatest variation in the 3 hours, 1500 cells. Thus the maximum error attributable to technic in this particular case is 47 per cent. However, the majority of the counts checked within a narrower range and the technical error in general where the total number of cells is larger may be depended upon not to exceed 10 per cent. The actual cell differences in the two counts each time are as follows:

400	250	500
690	650	0
700	250	25
160	100	225
		525

Obviously the smaller the number of cells the greater the percentage of error in the counting on the basis of the computations necessary in the present method of estimating total cells per c.mm.

At such points as the counts taken at 2.00 and at 2.45, where instead of the usual paralleling of the total cell change in the two pipettes the two counts diverge slightly, the average of the two, of course, gives a concept no nearer the true count than either of the extremes. Consequently such results admit of no analysis or interpretation. It is only in counts such as those at 3.45 and 4.00 where the changes are relatively marked and registered in both observations that one feels justified in trying to explain the variation on some basis other than technical. The practical importance of this point is, of course, only apparent in later cases with higher counts.

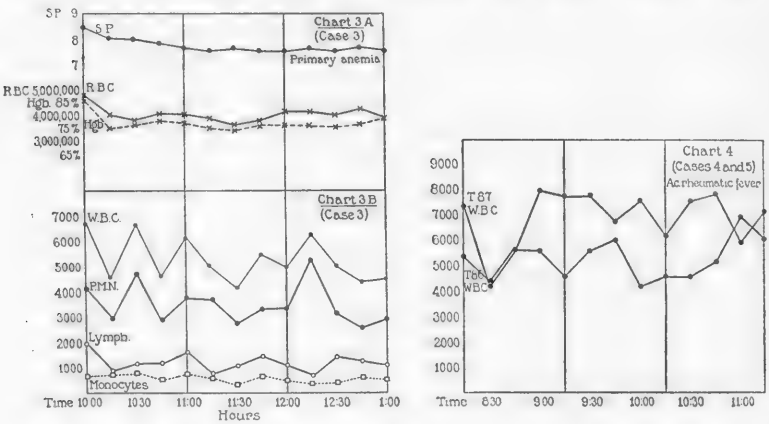
On Chart 1B are outlined the absolute numbers of myeloid, lymphoid, and monocytic cells, based on differential white blood cell counts on fixed preparations stained with Wright's stain, and the average total white cell counts. It will be noted at once that these three types of the white cells vary independently of each other. The increase of lymphocytes at 2.15 is responsible for the peak in the total white cell curve at that moment. The two elevations at 3.30 and 4.00 follow increases in the absolute numbers of myeloid cells, and for the 3 hour period there are four elevations roughly speaking for this group. The monocytes in this case illustrate the tendency to a 45 minute or hourly rhythm which we have noted in a number of cases. Monocytes also vary, rising and falling over periods of several days, as in typhoid fever and tuberculosis. This independent variation in the percentage and total numbers of the three types of white blood cells is the first evidence against a simple dilution explanation for the variations in the total cell count. It will be seen later and more strikingly in other cases. This case may illustrate also the fact that there is a tendency, as found in the normal, to a higher late afternoon white count since the only two counts over 2500 came after 4.00 p.m. The relative proportions of the three types of cells are approximately normal, *i.e.* there is not the relative lymphocytosis frequently seen in leucopenia. However, in so low a total count as is this, it is reasonable to expect the lymphocytes to influence more readily the total count at times. Thus the two groups of cells must be taken into account as mutually potentially influential on the total count in the leucopenic state.

Shaw (2) found, in single determinations in 116 healthy male adults, counted uniformly between 9 and 10 a.m., a percentage of lymphocytes equal to or exceeding the percentage of neutrophils in 10 persons, though subsequent observations in the same individuals usually gave the general excess of neutrophils. An interesting confirmation of the transitory nature of this lymphocyte preponderance was revealed in two of his studies on the diurnal tides, in which the percentage of lymphocytes temporarily exceeded the percentage of neutrophils for some hours. While the lymphocyte curves are not given in the paper, his analysis of the differential counts showed the fluctuations to be largely in the neutrophil group, so that it may be assumed that the transitory lymphocytoses were relative, due

rather to the decrease of granulocytes than to any marked increase in absolute numbers of lymphocytes (10).

Chart 2 (Case 2) is representative of a leucopenic range of from 2900 to 5300 white blood cells in a 3 hour period of observation on a case with the diagnosis of pernicious anemia. In this particular instance the counts were taken at 10 minute intervals to see if any marked difference in the nature and range of the fluctuations could be discerned. Four elevations alternate with lower counts between, two peaks coming within the 2nd hour. This is not an infrequent observation, even though the usual variations at 15 minute intervals over a long period show but one rise on the average each hour.

The second case of pernicious anemia with a relatively low white count showed a fluctuation of from 4000 to 6500 white cells during the 3 hours. Charts 3A and



3B graphically depict the relationship which was found to exist between the total white blood cells, red blood cells, hemoglobin, and serum protein. Two independent series of total white counts were made again for a check on the error of counting, though not charted. It will be seen at once that the white blood cells fluctuated much more widely than, and entirely independently of, the red blood cells, the hemoglobin determinations closely paralleling the latter. The serum protein dropped gradually during the 1st hour, thereafter showing only very slight variations, between 7.55 and 7.67 per cent. On first glance it would appear that the differences in all determinations between the first and second observations, 10.00 and 10.15 a.m., could be accounted for through blood volume increase with dilution and resultant lowering of serum protein, white and red blood cells, and hemoglobin. However, the percentage change when all are reduced to the same common denominator shows the white blood cell change to be 30 per cent, red blood cell 20 per cent, hemoglobin 12 per cent, and serum protein 8.2 per cent.

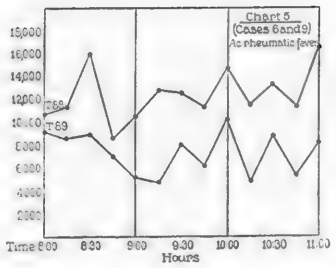


Chart 5
(Cases 6 and 9)
Acrheumatic fever
T68
T69
Time 8:00 8:30 9:00 9:30 10:00 10:30 11:00
Hours

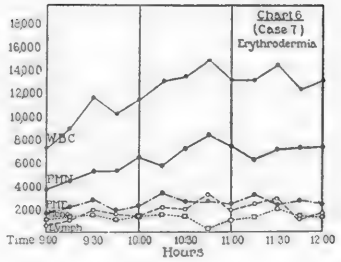
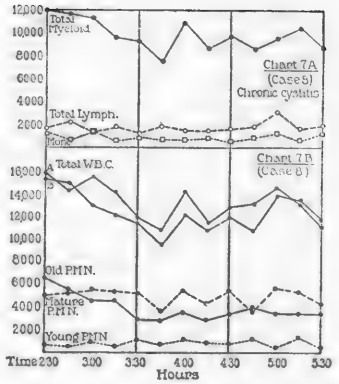
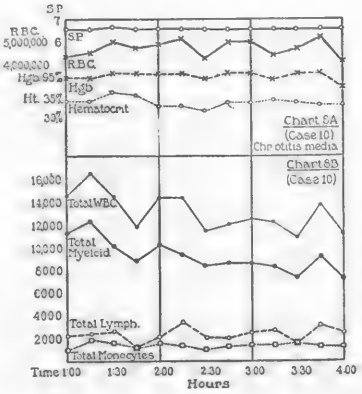


Chart 6
(Case 7)
Erythrodermia
WBC
PMN
Time 9:00 9:30 10:00 10:30 11:00 11:30 12:00
Hours


Total Myeloid
Chart 7A
(Case 5)
Chronic cystitis
Total Lymph.
Mono.
Chart 7B
(Case 8)
Total WBC.
Old PMN.
Mature P.M.N.
Young P.M.N.
Time 2:30 3:00 3:30 4:00 4:30 5:00 5:30
Hours


S.P.
R.B.C. 5,000,000
4,000,000
Hgb. 95%
Ht. 35%
38%
S.P.
R.B.C.
Hgb
Hematocrit
Chart 8A
(Case 10)
Chronic media
Chart 8D
(Case 10)
Total WBC
Total Myeloid
Total Lymph.
Total Monocytes
Time 1:00 1:30 2:00 2:30 3:00 3:30 4:00
Hours

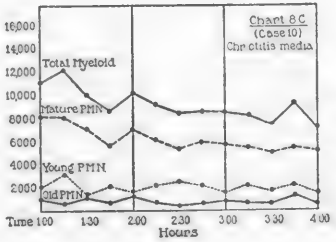


Chart 8C
(Case 10)
Chronic media
Total Myeloid
Mature PMN
Young PMN
Old PMN
Time 1:00 1:30 2:00 2:30 3:00 3:30 4:00
Hours

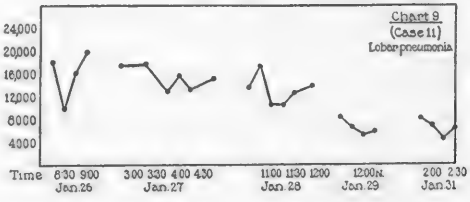


Chart 9
(Case 11)
Lobar pneumonia
Time 6:30 9:00 3:00 3:30 4:00 4:30 11:00 11:30 12:00 12:00N. 2:00 2:30
Jan. 26 Jan. 27 Jan. 28 Jan. 29 Jan. 31

Nevertheless, should such parallel changes in the same direction always occur in all the fluctuations, it would be justified to attribute small discrepancies in actual identical percentages to the error indisputably a part of each technical procedure. But this has never been found to be consistently the case. As in the instance in point, Chart 3A, the serum protein falls directly to a relatively fixed level and may be correlated with the fact that the patient was not on a fasting base line.

Chart 4 represents two series of white counts which might well serve as an example of the range of variation in individuals with a low normal total count, yet both patients had acute rheumatic fever, not yet treated, Case 4 having two active joints at the time of the count. Three elevations in the total counts may be noted in each series, during the 3 hour period. Chart 5 carries two acute cases under treatment, with the same diagnosis but with the counts in Case 9 definitely above the normal range, and each showing in the first 2 hours two peaks but in the 3rd hour two half-hourly peaks. With the recognition of the physiological fluctuations in the white cells, the influence of treatment (11), but the possible presence of activity without elevation in the total count, prognostic deductions based on small variations in the total white count in acute rheumatic fever should be conservative.

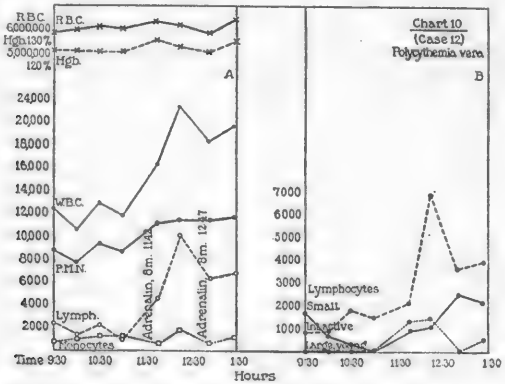
Leucocytoses.

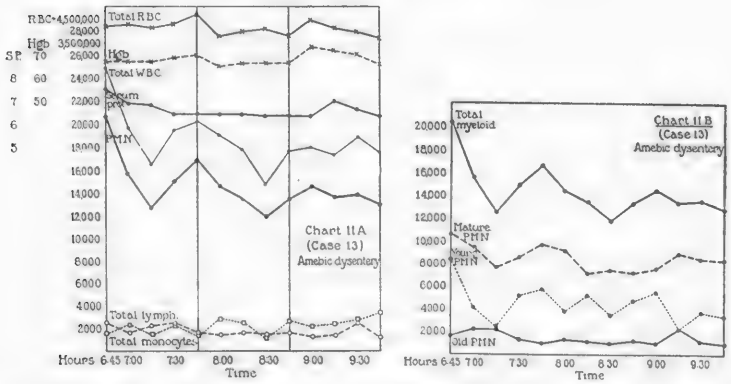
Chronic.—It is of interest to note the differences appearing in the chronic and acute leucocytoses. The chronic state is represented by Cases 8 and 10 as graphed in Charts 7A and B, and 8A, B, and C.

The case, No. 8, of chronic cystitis was running a white count between 9500 and 15,500, the chronic otitis media, Case 10, 10,800 to 16,500. There is a definite hourly rhythm in both cases, and the low points reached in each had they been single observations would have been equivocal in so far as a leucocytosis is concerned, particularly since they were chronic conditions and the adjustment of the bone marrow had been made on the new level. Charts 7B and 8C indicate the Arneth differentials which show a low level of young and old white cells, with the majority mature forms, even as is the case in the normal; in contrast, in the acute leucocytoses, the Arneth "shift to the left" is constant. Lymphocytes and monocytes are within their normal limits, the neutrophil group being entirely responsible for the elevation in total white count.

It will be seen strikingly again and again that particular uncomplicated pathologies stimulate independently one or another of the cell types and that the response of the organism is either limited entirely to this specific pathognomonic reaction or so predominantly to the one that the slight relative increase in the other types is obviously not a part of the specific response. Hickling (12) has pointed this out

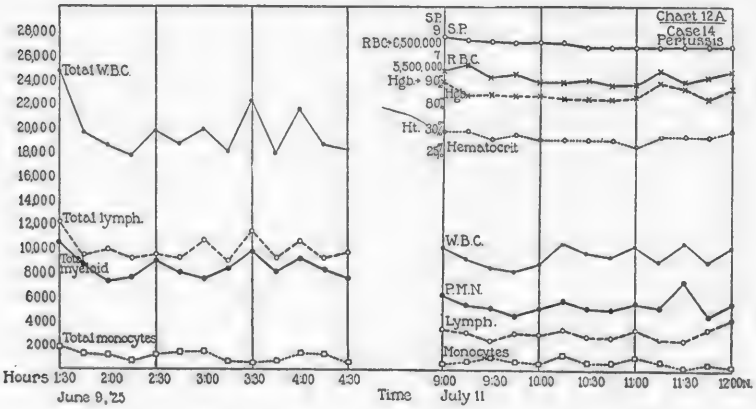
in a clinical and experimental study of toxic and chemical stimuli on the circulating leucocytes. He found a specific neutrophilic response to the presence of bacterial infection with the degree of postinfective lymphocytosis dependent upon the extent of tissue injury. In the

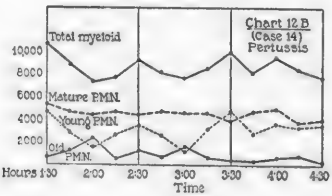




particular response as analyzed in the present series, the specific cell type maintains its original rhythm as will be seen in Chart 10A with lymphocytes elevated after adrenalin, in Chart 12A in which the lymphocytes are elevated in a case of pertussis, in Chart 6 in which there is an eosinophilia with erythrodermia, and in Chart 16A giving a neutrophilia typical of lobar pneumonia.

Acute Leucocytoses.—The group of acute leucocytoses secondary to infection are represented in six cases, two with diagnoses of lobar pneumonia, one with amebic dysentery, one with rheumatic endocarditis, one with lung abscess, and the last with streptococcus septicemia. All show the specific polymorphonuclear neutrophil increase with the "shift to the left" in the Arneth differential pattern, the younger neutrophils both showing a higher percentage than is found



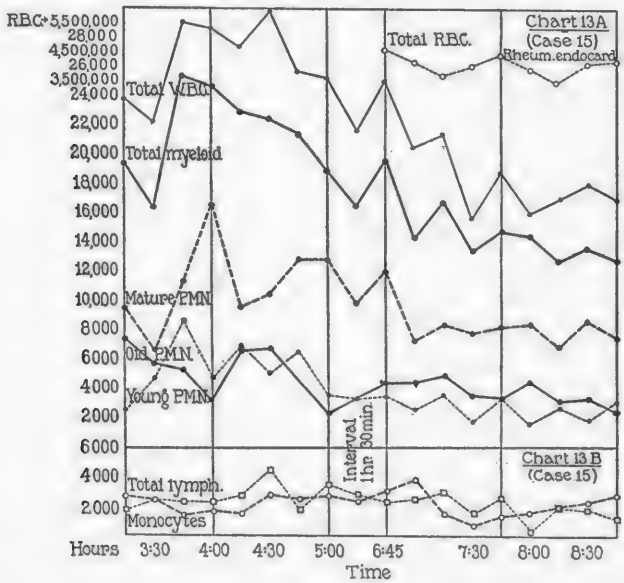


normally and the high points in the consecutive counts being seen to be a reflection of periodic increases in these forms.

Charts 11B, 14, and 15 indicate this particularly clearly. Chart 13A which combines an afternoon period with a later evening period of observation on the same day indicates the high late afternoon total count with a marked "shift to the left" of the Arneth differential and an evening count decidedly lower reflecting a lowered percentage of young cells. The lymphocytes and monocytes maintain essentially undisturbed total numbers throughout both series of counts, and the

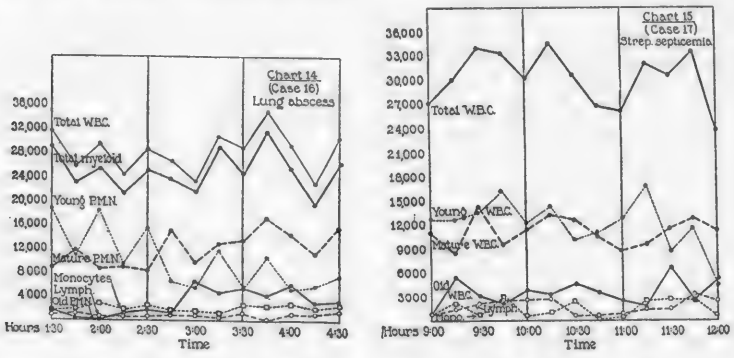
red cells during the later period show no consistent downward trend as do the white cells.

Chart 9 graphically presents the total counts done on consecutive days in a case first seen on the evening of the 3rd day of the disease. The patient gave a history of sudden onset of symptoms typical of lobar pneumonia occurring at 9.30 a.m. January 24. He arrived at the Hospital on the 26th without definite physical signs in the chest, with a temperature of 102.5°, pulse 100, and respirations 35. The white count on admission was 8000. It happened to be a period when influenza, rather than lobar pneumonia, was prevalent, so that the low total white count and physical findings in spite of the history seemed to coincide better with

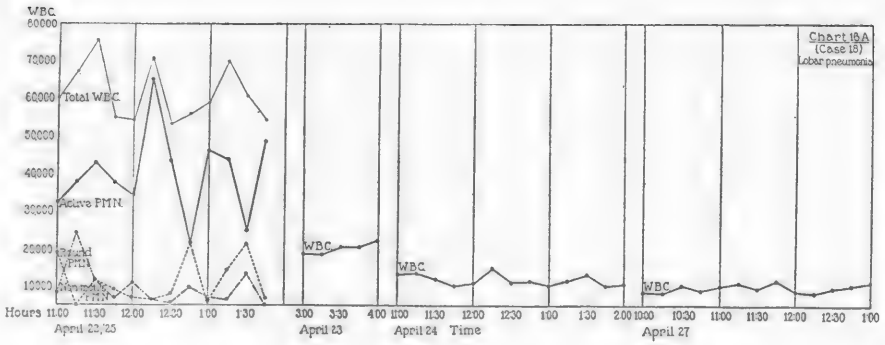


the former diagnosis. Within an hour the four counts taken at 15 minute intervals and recorded on Chart 9 had been taken. It will be seen that one of the four counts was 10,000 but that the other three were all above 16,000, one going to 20,000. Both the 8000 and the 10,000 total white blood cell counts were in all probability accurate determinations of the numbers of these cells in the peripheral blood at the moment those samples were taken, but represented only the lowest swings of an arc, which was periodically at least, indicative of a definite pyogenic stimulation. In this case there was never the marked leucocytosis seen in Case 18, but a lobar consolidation developed and a typical crisis came on the 5th day with the fall to normal in the total white count as shown under date of January 29 on the chart.

Case 18 (Chart 16A) presented the typical history and picture of a severe, uncomplicated lobar pneumonia, with complete consolidation of the right lung, temperature, at 1.00 p.m. April 22, the day of the first count, 106°, pulse 150, respirations 50. The crisis came during this afternoon, the 4.00 p.m. temperature being 100° and the 8.00 p.m. temperature 98°. The first 3 hour count, therefore, represents the maximum mobilization of the cellular defense in this patient just prior to, and almost coincident with, his crisis. It will be seen that there were three high points, one each hour, at which the total count reached or surpassed 70,000 per c.mm., but that seven of the twelve counts were between 50,000 and 60,000. The supravital differential counts revealed a high percentage of actively motile neutrophils, particularly at the moments of the highest counts, with the round and "non-motile" forms, indicating injury and cell death, relatively few in number, except at the periods of lowest ebb in the count. This is in correlation with the periodic "non-motile showers" (1) found in the counts on normal in-



dividuals. The same prognostic significance we believe is probably attached to this observation as Pons and Ward (13) attribute to the leucocyte fragility test in pneumonia, *i.e.* the larger the percentage of living cells the better the prognosis. Mauriac and Moureau (14) also analyze the curves of fragile cells most instructively. Chart 16B shows peaks in the young and mature neutrophils (Arneth differential on fixed films) corresponding with the peaks of total cells and with the high points in numbers of active cells in the supravital preparations. Chart 16C shows a normal number of lymphocytes, the pneumonic process having had no influence on this strain of cells; the monocytes show fluctuations from a normal level to a considerable increase over the usual percentage of these cells in normal blood (15). Chart 16A includes the counts taken on successive days following the crisis, showing totals ranging from 18,000 to 22,000 during only five observations on the 23rd, 10,000 to 14,000 on the 24th, and on the 27th, 6000 to 12,700. The last two periods, as the first, show the tendency toward an identical hourly fluctuation in the total cells. Whereas the polymorphonuclear neutrophils during


WBC
Total WBC
Active PMN
Old PMN
Young PMN
Monocytes
WBC
WBC
WBC
Chart 16A
(Case 16)
Lobar pneumonia
Hours 11:00 11:30 12:00 12:30 1:00 1:30 3:00 3:30 4:00 11:00 11:30 12:00 12:30 1:00 1:30 2:00 10:00 10:30 11:00 11:30 12:00 12:30 1:00
April 22, 25
April 23
April 24
April 27
Time

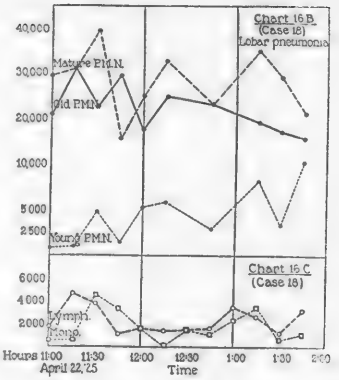


Chart 16B
(Case 16)
Lobar pneumonia
Mature PMN
Old PMN
Young PMN
Chart 16C
(Case 16)
Lymph.
Mono.
Hours 11:00 11:30 12:00 12:30 1:00 1:30 2:00
April 22, 25
Time

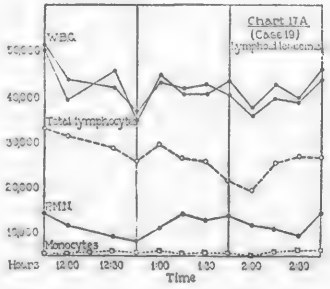


Chart 17A
(Case 19)
Lymphoid leucemia
WBC
Total lymphocytes
PMN
Monocytes
Hours 12:00 12:30 1:00 1:30 2:00 2:30
Time

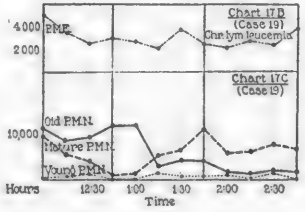


Chart 17B
(Case 19)
Chr lym leucemia
PMN
Chart 17C
(Case 19)
Old PMN
Mature PMN
Young PMN
Hours 12:30 1:00 1:30 2:00 2:30
Time

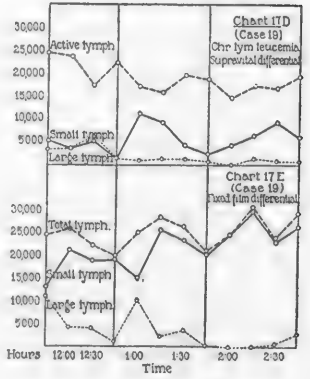


Chart 17D
(Case 19)
Chr lym leucemia
Supravital differential
Active lymph.
Small lymph.
Large lymph.
Chart 17E
(Case 19)
Fixed film differential
Total lymph.
Small lymph.
Large lymph.
Hours 12:00 12:30 1:00 1:30 2:00 2:30
Time

the period of the crisis were averaging from 89 to 98 per cent of the total count, on the 2nd and 3rd days thereafter they were 77 to 90 per cent, and on the fourth count 60 to 75 per cent. Thus the last counts made on the 5th day after the crisis showed the return to an entirely normal range of total white cells with the normal differential both as to supravital and Arneth classifications except for some increase in the monocytes, 8 to 14 per cent. Hickling (15) has just made a very interesting analysis of the monocytes in pneumonia, correlating their late rise in the course of the disease with resolution, and drawing certain prognostic conclusions from their curve from day to day.

Such an analysis, as here attempted, of successive white blood cell counts during a leucocytosis might indicate a periodic delivery of new cells from the hemopoietic organs, the intervening intervals of decreasing total circulating neutrophils being representative of their constant loss to the tissues at the site of the disease in their functional capacity of local cellular defense. The importance to the surgeon of the recognition of these rapid fluctuations in cases of an acute abdominal condition, with the advisability of frequently repeated counts when the diagnosis is in doubt, is evident. The degree to which the multiplication and maturation, of the particular cell type required for the body defense in a specific disease, may be speeded up is an approximate index of the cellular factor in the complex of the "resistance" of that individual (16, 17).

Lymphocytosis.

Case 14 (Chart 12A) presented an equivocal picture on admission to the Hospital with few symptoms, a low grade fever, a cervical adenopathy, and a moderate leucocytosis. The differential count showing a decided lymphocytosis was suggestive and the eventual development of the characteristic cough established the diagnosis. The 3 hour period of 15 minute counts in this case of pertussis reveals a curve of fluctuations in the lymphocytes quite distinct from that of the leucocytes. Because the lymphocytes are in total numbers actually in excess of the neutrophils the fluctuations in the former tend to be reflected in the total count. It will be seen that the usual hourly rhythm of the myeloid cells is maintained, that the peaks are made largely by the influx of young cells (Chart 12B), and that the lymphocytes fluctuate with each count, or on a half hourly swing, as was found for the lymphocytes in normal counts (1). The monocytes, eosinophils, and basophils are all within normal limits. 1 month after the first series of counts a second period of observation was made just prior to the patient's discharge from the Hospital. It will be noted that the total count had subsided to within normal limits, the lymphocytes having returned to normal percentage and number, and

the neutrophils once more reflect their normal predominance in the curve of the total cells.

Chart 10 is presented in further support of the independence, observed so frequently, in the specific response of different cell strains. The leucocytosis following the administration of adrenalin is essentially a lymphocytosis in which the rise is first of the large young forms and then in the mature intermediate and small lymphocytes (10B). This curve is representative of three similar cases, one being a normal individual. It would appear that the change in the white cells under these conditions is not the reflection primarily of a blood volume or redistribution phenomenon, but rather the result of a direct influence on the lymphocyte supply, or its physiological control. Rous (10) found in dogs that the tissues producing lymphocytes are "set" at a rate of activity definite for the individual but subject to wide variations under the influence of changing physiological conditions. Inasmuch as such physiological stimuli are always potentially interactive, in the normally functioning individual periodicity in the delivery of lymphocytes may be assumed on the basis of the experimental work.

It is difficult to escape the impression that there are independent physiological controls for maintaining the relative, normal levels of the different types of white blood cells (14, 18), just as there is a body, growth-limiting, mechanism, a heat-regulating center or mechanism, and a cardiac regulatory mechanism. The readjustment of the control of the normal level for a specific cell strain, while not influenced appreciably by body posture, vasomotor instability, or variation in local distribution (19, 20), nevertheless must be subject to many chemical and toxic influences (12, 16, 21, 22, 23), and may be independent of the actual supply of the particular cells, as was indicated in the case of Minot and Isaacs (24) in which, following a large transfusion of lymphoid leucemic blood into a patient with marked lymphopenia, the original low level of lymphocytes was regained within $2\frac{1}{2}$ hours without evidence of cell destruction and without constitutional reaction. This observation indicates an altered regulatory mechanism in this patient for maintaining a low level of circulating lymphocytes independent of the supply or of a primary lymphocytolytic factor. However, in the individual physiological mechanism, it is probable that the altered control factors are

usually interactive with the formation and maturation processes for the particular cells affected and respond to the general law of supply and demand. Obviously the above discussion does not relate to true cytolytic effects on the mature circulating cells of any strain, the reaction to which is usually a compensatory hyperplasia in the attempt to establish an equilibrium at the new level of turnover in the particular cell type, and is simply the effort to maintain the normal established level for the physiological functioning of that cell.

The Leucemias.

When a study of the variations in the peripheral blood in the leucemias is attempted, however, it appears that the laws which seem to govern formation, maturation, and delivery of the cells involved, under normal and other pathological conditions, no longer hold. No evidence in Case 19 (lymphoid leucemia), or in Case 20 (myeloid leucemia), of the periodic rhythmicity more or less characteristic of the curves of lymphocytic and myeloid cells discussed above, can be found, as perhaps could hardly be expected when the presence of large numbers of immature forms in the peripheral blood indicates an entirely disordered mechanism of maturation and delivery.

The 3 hour period of observation in the case of chronic lymphoid leucemia cutis gave a range of fluctuation in the total count from 35,000 to 52,000, and the lack of conformity in the curves of lymphocytes and neutrophils and the unaffected status of the monocytes can be seen (Chart 17A). However, it is impossible to establish any periodicity in the total lymphocyte curve within the time period recorded. Charts 17D and E indicate a relative relationship between the young immature cells and the total lymphocyte curve, both falling during the first 2 hours, and Chart 17D, compiled from the supravital differential counts, shows the large number of lymphocytes showing normal motility. The neutrophils maintain an undisturbed hourly elevation and fall (Chart 17A), and show an Arneth differential count within normal limits (Chart 17C). The increased eosinophils in this case are shown on Chart 17B, the rhythm being an hourly one though independent of the neutrophil fluctuations. The eosinophilia in the case of erythrodermia (Chart 6) is quite comparable and probably this specific response in both cases is related to the respective skin involvements. Cioni (25) discusses the mechanism of eosinophilia relative to the nature of the specific chemical stimuli altering the bone marrow supply of these cells, and believes that in the case of infectious skin conditions it may be due either to the metabolic products of the parasite or to products of the destruction of the epithelium, or to both.

The striking thing in the analysis of the fluctuations in the case of myeloid leucemia, with a range in the total count from 200,000 to 284,000 during the 3 hour period of observation, was the inverse relationship between the mature neutrophils and the myelocytes (Chart 18A). Since it was a chronic case, the myeloblasts were relatively few in all counts. With every increase in the number of mature leucocytes there was a corresponding fall in the myelocyte level, and *vice versa* (26). This was particuarly striking in the curves of neutrophilic myelocytes and the young mature neutrophils (Chart 18B), an increasing divergence in these two curves being manifest toward the end of the period, the later total counts reflecting this rise in the myelocytes directly. The independent fluctuations of eosinophil, basophil, and neutrophil myelocytes show only the disordered chaos of the myeloid hyperplasia of the bone marrow, though the mature cells still show some semblance of a periodic fluctuation with three peaks during the 3 hours. In the leucemias it would seem that all regulation both as to primary formation and delivery and as to concentration of the cells in the peripheral blood was gone.

The Bone Marrow.

Any attempt at a quantitative estimation of the various cellular elements making up the bone marrow must, in the very nature of the survey, always be open to many questionings: the limitations inherent in any technic used, the fallacies inescapable in trying to draw any general deductions from even 1000 or 2000 cells counted out of the multiple millions present in any functioning bone marrow, the much debated questions of identification and classification of immature forms, the many factors known and unknown affecting hemopoiesis in any particular individual, all of these, and more, make it necessary to be very conservative in the drawing of deductions from any one limited series of observations or in trying to compare the figures obtained from different investigators.

Using the supravital and fixed technic as outlined at the beginning of the paper, we have found a reasonable degree of conformity in our counts, and in so far as any constant procedure can assure comparable results, the findings, as contained in Table III, may be so analyzed. Only very general indications, which might be most readily admitted as based on indisputable cellular identifications, will be attempted.

In the six cases under 8 years of age the relatively high percentage of lymphocytes will be noted, with none or very few mature polymorphonuclear leucocytes. This is in conformity with the well recognized relative lymphocytosis in children. In the presence of infection

TABLE III.

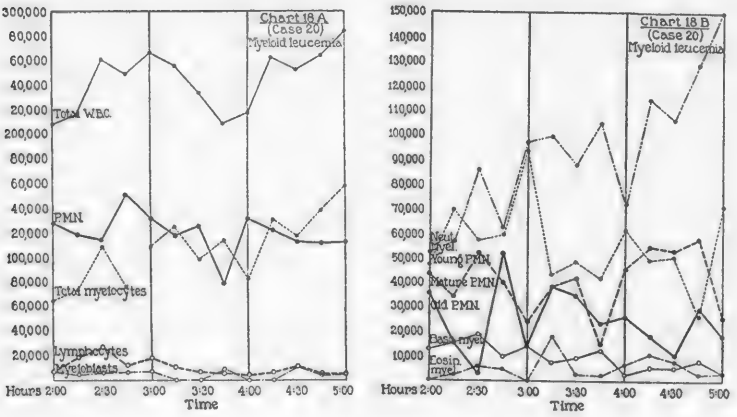
Human Bone Marrow Differentials Based on Count of 1000 to 2000 Cells.

Diagnosis	Polys.	Myelocytes			Myeloblasts	Prim. cells	Clasmatocytes	Monocytes	Megacaryocytes	Lymphocytes	Lymphoblasts	Eosin.	Basoph.	Megaloblasts	Normoblasts	Unclass.	Age
		Neut.	Eosin.	Basoph.													
																	mos.
1. Acute dehydration	0.	35.2	1.4	0.	5.9	0.	0.3	9.4	0.4	38.9	0.	2.4	0.	0.1	5.2	0.8	4
2. " "	0.	31.1	2.2	0.6	5.	0.4	1.2	4.2	0.2	44.9	0.	0.	0.	0.	9.1	1.1	5
																	yrs.
3. Normal (accident)	15.3	31.	0.37	0.	1.5	0.81	0.27	4.5	0.45	37.3	0.	0.54	0.	0.	5.7	1.1	4
4. Diphtheria	4.8	56.4	1.6	0.9	1.5	0.2	0.9	3.1	0.6	27.7	0.	0.	0.	0.	1.5	0.8	6
5. Pertussis-bronchopneumonia	0.6	61.8	0.6	0.	1.	0.	0.3	2.1	0.4	28.3	0.	0.	0.	0.1	3.5	1.3	8
6. Bronchopneumonia (vertebral bone marrow)	0.9	56.9	3.	0.1	4.2	0.5	0.6	3.	0.1	29.1	0.	1.	0.	0.	0.6	0.	7
7. Normal (accident)	55.4	18.8	4.1	0.	1.1	1.1	0.	1.6	0.1	10.8	0.	0.6	0.1	0.1	5.4	0.8	28
8. " "	10.5	53.1	0.8	0.	11.9	0.4	0.1	3.5	0.	15.	0.	0.2	0.	0.	4.1	0.4	29
9. " "	13.7	54.9	0.1	0.	1.7	1.	0.	0.5	0.1	14.7	0.	0.	0.	0.	13.	0.3	30
10. Acute cerebral hemorrhage	19.7	40.5	0.9	0.3	5.	0.3	1.	5.2	0.5	9.1	0.	0.7	0.	0.	14.2	2.6	72
11. Lobar pneumonia	19.2	63.5	0.6	0.	4.6	0.2	0.3	1.1	0.	8.8	0.	0.	0.	0.	1.7	0.	28
12. " "	90.	5.	0.1	0.	1.9	0.	0.1	2.	0.1	0.	0.	0.8	0.	0.	0.	0.	60
13. Empyema (biopsy rib)	47.7	30.3	0.8	0.	8.5	0.7	0.2	0.8	0.4	6.3	0.	0.3	0.	0.6	3.	0.4	42
14. Lung abscess	46.7	33.3	1.	0.6	2.7	0.4	0.	2.	0.	6.2	0.	0.2	0.	0.8	3.8	0.7	32
15. Subdiaphragmatic abscess	65.4	15.	1.7	0.	2.6	0.3	0.	0.9	1.1	7.4	0.	1.7	0.	0.1	3.1	0.7	38
16. Empyema (biopsy rib)	46.	30.6	1.9	0.	2.6	1.4	0.	1.1	0.2	10.1	0.1	0.3	0.	0.4	4.7	0.6	38
17. Postpartum infection	27.8	37.8	0.3	0.	3.5	0.1	0.	6.8	0.4	15.	0.	1.1	0.	0.	6.8	0.4	44
18. General sepsis	5.7	65.1	0.3	0.	1.4	0.1	0.1	13.8	0.2	8.3	0.	0.	0.	0.2	4.1	0.3	36
19. Cardiovascular lens. Pneumonia	9.3	71.	0.4	0.2	1.1	0.2	0.1	4.7	0.2	9.3	0.	0.	0.	0.	2.1	1.4	59
20. Cirrhosis of liver	4.4	75.1	0.5	0.2	2.5	0.6	5.2	3.4	0.7	5.	0.	0.5	0.	0.	1.7	0.2	61
21. " " "	1.6	52.7	1.6	0.5	8.	3.1	0.6	8.1	0.1	3.6	0.	0.1	0.	0.	17.7	3.3	46

22. Cirrhosis of liver	6.	58.7	1.1	0.	0.	1.3	0.7	8.7	0.4	20.3	0.	0.1	0.	0.	0.8	0.	57
23. " " " (infection)	26.3	50.1	0.6	0.1	2.1	1.	1.4	1.3	0.	13.1	0.	0.6	0.	0.	3.	0.4	49
24. Pernicious anemia	7.6	62.9	1.7	0.1	4.7	0.6	5.5	6.8	0.6	5.8	0.	0.5	0.	0.6	1.	1.6	62
25. " "	2.9	54.6	4.1	2.6	0.3	0.7	5.5	4.3	0.	20.1	0.	1.5	0.2	1.9	1.	0.5	61
26. " "	0.5	14.3	30.3	0.9	5.3	0.	6.7	12.6	0.	10.3	0.	0.	0.	10.6	3.2	0.8	56
27. " "	6.8	56.	1.6	0.2	1.6	0.5	5.6	7.1	0.	16.8	0.	0.7	0.	1.5	1	0.6	58
28. " "	8.1	12.8	10.6	1.6	19.7	0.8	3.4	13.1	0.	11.6	0.	0.6	0.2	14.4	2.1	1.	46
29. Acute miliary tuberculosis	31.1	9.42	0.38	0.09	2.66	0.19	0.	21.9	0.	32.	0.	0.66	0.	0.	1.42	0.57	36
30. Lymphatic leucemia. Cirrhosis of liver	0.	0.	0.	0.	0.	0.	0.	4.8	0.	0.	93.	0.	0.	0.	0.4	1.8	48
31. Banti's disease	5.1	42.4	2.2	0.	2.	0.3	8.2	5.6	0.3	27.6	1.3	0.8	0.	0.3	2.8	1.1	48
32. Carcinoma of pleura	7.3	42.	14.7	0.9	3.	0.3	5.2	1.3	0.5	13.6	0.	1.8	0.	0.	5.4	3.	52

(Cases 4, 5, and 6) the myeloid cells were increased though the response was not to the extent seen in adult cases (27).

There are four cases in the series (Cases 3, 7, 8, and 9) which have been considered to present normal bone marrow pictures, inasmuch as they were medicolegal cases of sudden accidental death in supposed healthy young adults (one child of 4 years). It is only desired to call attention to the relationships found between mature polymorphonuclear neutrophilic leucocytes and the neutrophilic myelocytes. It may be granted that the distinction between cells with characteristic multilobed nuclei and their full complement of specific granules and those with oval single nuclei and relatively fewer specific granules is



possible of ready appraisal by any worker familiar with the blood. Thus while the total myeloid percentage in the three adult cases showed close agreement, 74 per cent, 64 per cent, and 68 per cent, in Case 7 there were 55 per cent segmented forms and 19 per cent myelocytes, in contrast to Cases 8 and 9 with only 10 and 13 per cent mature cells and 53 and 55 per cent myelocytes, respectively. Schilling (28) and Benzler report a cytological study of the bone marrow from ten normal healthy men, just shot, in which the myeloid elements ranged from 39 to 43 per cent, eosinophils from 1.1 to 4.6 per cent, and normoblasts from 31 to 42 per cent. The peripheral blood in these cases showed neutrophils ranging from 32 to 74.5 per cent, and

in the bone marrow differentials, the myelocytes varied between 34 and 47 per cent, the metamyelocytes between 34 and 56 per cent, and the segmented nuclear cells between 5 and 22 per cent, but there is no relationship expressed in the individual cases. In the present series, with the technics used, normoblasts were found in much smaller relative percentages than those given above by Schilling, *viz.* 4 to 13 per cent in the normal cases and from 0 to 17.7 per cent in the pathological cases. The latter figures are more in accord with those reported in a small series of pathological cases by Doan (7) with the supravital technic, in which the normoblasts were found in percentages of from 4 to 22. A study of the fixed sections of bone marrow, except perhaps in certain severe secondary anemias and pernicious anemia, gives a definite impression of the predominance of myeloid metaplasia over erythroid foci in contrast to the relative numbers of the respective definitive cells in the peripheral blood. This must be related to the more rapid turnover in the white cells, the red cells under most conditions probably surviving for a much longer period; also the entire functional activity of the red cells is dependent upon their circulation within the vascular bed, whereas the circulation is simply the means of communication for the white cells from source of supply to the local points of activity in the body outside the blood stream. Certainly with the supravital technic, which has now been used in many scores of surveys of bone marrow from experimental animals (29, 30), and with the fixed technic as here used, the nucleated red cells have been far more often below 20 per cent of the differential than above.

In two instances of lobar pneumonia (Cases 11 and 12) the total myeloid cells in the bone marrow were 82.7 and 95 per cent respectively, but in the former there were 19.2 per cent mature cells and 63.5 per cent myelocytes, whereas in the latter the mature cells were 90 per cent and myelocytes 5 per cent.

A comparison of the first two columns of Table III straight through shows the reciprocal relationship maintained between the percentages of mature and immature neutrophils. When taken into account in the presence of the observed periodic fluctuations in the total white blood count in the peripheral blood, as presented in the first part of this paper, a tenable hypothesis of the periodic maturation of myelo-

cytes into polylobed, motile leucocytes may be conceived. The particular moment at which the process of manufacture is stopped would give for a given area either a majority of mature neutrophils or the reverse according to the phase of maturation at that point at that time. This has been particularly striking in the experimental study of the bone marrow after specific stimulation of the maturation of the neutrophil group by sodium nucleinate and similar substances producing a peripheral leucocytosis (29). In bone marrows relatively hypoplastic, so that the two processes of red and white cell formation may be analyzed separately, it is striking to find always the cells in any one focus or area all at the same stage of maturation (30, 31, 32). Thus, it would seem from all the evidence at hand that the white cells, leucocytes, lymphocytes (33), and monocytes (34, Simpson "macrophage showers") are constantly leaving the blood stream, and that the supply is primarily dependent upon a more or less periodic maturation and delivery of granulocytes from the bone marrow, lymphocytes from the thoracic duct (10, 35), and monocytes and clasmatocytes possibly from the spleen. It is not believed that other factors are negligible as affecting the total numbers of circulating cells, but that the factors mentioned are important in the cellular economy of the body seems probable.

In view of the recent attention attracted to the cellular aspects of experimental and clinical tuberculosis (36, 37) it is of interest to note the one case (No. 29) of miliary tuberculosis in which the monocytes in the bone marrow were 21.9 per cent and the lymphocytes 32 per cent. It has been shown (36) that the typical epithelioid cell of tuberculosis, when stained with one of the Romanowski stains, reacts with a characteristic faint eosin tint in the cytoplasm near the *Hof* of the nucleus where the rosette of neutral red bodies is found in the supravital preparation. In this case these cells could not be mistaken, and though listed as monocytes the majority of the cells were true epithelioids. In no other case were monocytes found in as high percentages in the bone marrow, and the lymphocytes only in the cases within the first decade of life.

The five cases of pernicious anemia, Nos. 24, 25, 26, 27, and 28, show the well recognized (7, 38) increased percentages of clasmato-

cytes, again a cell type easily recognized through its marked capacity for phagocytosis.

SUMMARY.

In a study of twenty clinical cases with a wide range of diagnoses, repeated total counts of the white cells at 15 minute intervals reveal a large fluctuation at various levels comparable to that found for the normal (1, 2). The granulocytes seem to follow a more or less hourly rhythm, the most marked shift to the left in the Arneth pattern and the moment of greatest percentage of motility coinciding with the peaks.

The independence found existing between the peripheral blood concentrations of individual strains of white cells and the red cells, as determined by total and differential counts, their differential response to pathological and pharmacological stimuli, and their normal relative relations, all indicate some separate physiological mechanism of control for each type of cell, either working through, or independently of, their sources of origin.

The many factors to which the circulation of the blood, as such, is subject, the complexity of the influences on origin, maturation, delivery, longevity, and destruction of each cell group, the limitations inherent in the present involved, indirect technics of counting, combine to make any single observation subject to grave misinterpretation. The value to the clinician must come in repeated observations, at times when the diagnosis or a therapeutic procedure is in doubt, at frequent intervals, at other times over longer or shorter periods, but always with the relation between consecutive counts, rather than the absolute values, the important point for consideration.

Both the red and the white cells probably change their relative concentrations in the peripheral blood from time to time over a considerable range that is quite within normal physiological limits, so that, in theoretical considerations and in practical functional estimations, a zonal concept with adequate individual extremes should always be kept in mind for both physiological and pathological states.

A cytological analysis of thirty-two bone marrows from human biopsy and autopsy material shows the striking reciprocity found to

exist between the myelocytes and the mature polymorphonuclear leucocytes. This, together with the observed focal uniformity of maturation found in bone marrow, and the periodicity of the fluctuations of the neutrophils in the peripheral blood, leads to the formulation of the hypothesis of a constant functional withdrawal of granulocytes from the peripheral blood with a periodic delivery of new cells from the marrow, which in leucopenia and in leucocytosis represents a depression or a stimulation, respectively, of the normal mechanism. The nature and degree of the response are an approximate index of the cellular factor in the complex of the "resistance" of the particular individual.

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